Antioxidants in fruits and vegetables

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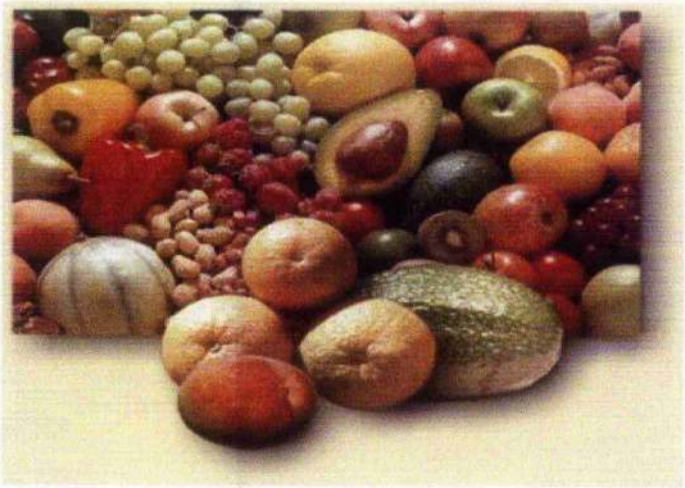

A thesis submitted to the University of Glasgow for the degree of Master of Science (M.Sc.)

Institute of Biomedical & Life Sciences
University of Glasgow

July 2004

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ABSTRACT

Diets rich in fruits and vegetables are associated with a reduced risk of cardio-vascular disease and some cancers. It has been more difficult for researchers to identify the specific component(s) of these diets responsible for the "protective" effects. Attention has recently been focused on phenolic and polyphenolic components, which may have biological activity in humans, by acting as antioxidants. Levels of such compounds in individual fruits and vegetables are affected by soil, climatic conditions, physiological stress under which they are grown or even agricultural methods.

In particular, organic products are thought to be healthier than their conventional equivalents. To date, few studies have investigated the impact of various agricultural practices on the level of phenolic compounds present in the crops. In this study, the total phenolic content, as well as the antioxidant activity and the vitamin C content of different fruits and vegetables were measured in both organically or conventionally grown crops, available from a local market. The present study also compared the effect of the consumption of conventionally and organically produced kiwi fruits on the plasma antioxidant activity of humans. Despite the interest of consumers in organic products, the findings from this project did not show any consistent differences in antioxidant capacity, vitamin C or phenolic content between conventional and organic crops, available to consumers at local supermarkets.

The number of natural polyphenols has been estimated to be several thousands, as they occur in plants as conjugates. In the present study, we tried to determine, which of these compounds contributed to the antioxidant capacity in a range of 17 commonly consumed fruits and vegetables, which may be the basis for the beneficial effect of these foods. By combining three different techniques - HPLC, MS and on-line measurement of antioxidant activity - we could identify and quantify the bioactive compounds (vitamin C, polyphenols) present in a range of fruits and vegetables and determine their individual contribution to the total antioxidant activity. Results showed that the nature and the occurrence of antioxidants vary markedly

among plant species; the total antioxidant activity is due to the cumulative effects of different compounds rather than any individual compound.

Identification of polyphenols in fruits and vegetables is important in regard to their potential health effects. However, it is also necessary to determine if they are absorbed, metabolized in human body, and whether the dietary components and their metabolites provide any benefit within the gastrointestinal tract. In the present study, we investigated the absorption and excretion of anthocyanins and proanthocyanidins: a healthy subject with an ileostomy was fed 200 g of redcurrants and then provided plasma, urine and ileostomy fluid for analysis. This allowed an excretion profile to be obtained, giving a time span for the absorption of redcurrant polyphenols. Following the consumption of redcurrants, we could see that prodelphinidins were poorly absorbed (as 86% were recovered in the ileal fluid), and their potential action was, thus, restricted to the intestine, where they could act as antioxidants. Quantitative analysis of anthocyanins in urine collected over a 24 h period after ingestion of redcurrants accounted for only 0.02% of the amount ingested. No anthocyanins were detected in plasma. However, only 35 % of the ingested dose of anthocyanins was recovered in the ileal fluid, suggesting 65 % of the anthocyanins were transformed and/or absorbed. Further work would be required to identify the "missing" anthocyanins and determine their fate and their potential health effects within the body.

AUTHOR'S DECLARATION

The composition of this thesis and the work described within it was carried out entirely by myself unless otherwise cited or acknowledged. Its contents have not previously been submitted for any other degrees. The research for this thesis was carried out between August 2002 and July 2004.

Signed.... .....

Alexandra DEGENEVE

July, 2004

INDEX

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

To Alan for supervising my work, but also for all the good wine and dishes tested in his place over the last 2 years

To my sweet daddy (I prefer not to mentioned his name, not only because he could be in trouble with T., but also because it is not something I am very proud of!!!) for having mentioned to me a possible placement here: I know it was because you were drunk, but I should be in the same state when I accepted to come back, and support again your bad jokes about froggies…I should warn everybody your kind of humour is very contagious and I caught it!

To Mandie for dealing with my bad english and correcting my thesis with a lot of patience

To Gina for her permanent positive attitude and happiness, even when she had to deal with a lot of personal problems

To everyone in the lab, past or present, and especially Alan, Alison, Asa, Aurélie, Bill, Catherine, Cyril, Daniele, Gina, Indu and Mandie for all support and the good moments inside or outside the lab. You have made my time full of fun during the last 2 years

To my mum and dad for their support. Even if they still don't know what I was really doing here, they certainly understand how it was pleasant to be there with all of you, as they enjoy themselves visiting Scotland during their holidays.

Last but not the least to my beloved boyfriend Laurent for always being there, despite the long distance between us, and for keeping my life in expectation.

ABBREVIATIONS

ABTS	2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
$ABTS^{\cdot+}$	2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation
AH	Acid hydrolysis
amu	atomic mass unit
AOX	Antioxidant activity
CHD	Coronary heart disease
DNA	Deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDTA	Ethylene-dinitrilo-tetra-acetate
FRAP	Ferric reducing antioxidant power
GA	Gallic acid
HCl	Hydrohloric acid
HPLC	High performance liquid chromatography
i.d.	Internal diameter
ISF	In source fragmentation
LC-MS	Liquid chromatography mass spectrometry
LDL	Low-density lipoprotein
λmax	Absorbance maxima
MeOH	Methanol
MS	Mass spectroscopy
$[M-H]^-$	negatively charged molecular ion
$[M+H]^+$	positively charged molecular ion
NO	Non organic
NMR	Nuclear magnetic resonance
m/z	mass to charge ratio
PDA	Photodiode array
SE	Standard error
O	Organic
TEAC	Trolox equivalent antioxidant capacity
TPTZ	2,4,6-tripyridyl-s-triazine
t_R	retention time
UV	Ultraviolet
v/v	Volume/volume

Chapter 1 Introduction

There is a growing awareness that diets rich in fruits and vegetables are associated with lower risk of diseases such as coronary heart disease or cancer (Hertog *et al.*, 1992, 1993). Current diet advice suggests consumption on a daily basis of at least five portions of fruits and vegetables. Nonetheless, it is more difficult to identify the specific components responsible for these properties. In addition to vitamins, minerals and dietary fibre, fruits and vegetables are a source of phenolic and polyphenolic compounds that act as antioxidants and which may have biological activity in humans. The number of natural polyphenols has been estimated to be several thousands, and their nature or levels vary from plant to plant (Manach *et al.*, 2004). But which compounds in particular are the main contributors of the *in vitro* antioxidant activity? Does production method (organic or intensive agriculture) affect the phenolic content? In view of their potential effects in human, bioavailability is also considered to be an important issue.

1.1. Introduction to phenolics

Phenolics are secondary plant metabolites, with one or more hydroxyl groups attached. Their aromatic hydroxylated structure is believed to be responsible for their antioxidant activity (Simonetti *et al.*, 1997). Phenolics range from simple, low molecular weight, single aromatic-ringed compounds such as gallic acid to the large and complex tannins. They are synthesised via the shikimate and phenylpropanoid pathways (see Figure 1.1). They are commonly found conjugated to sugars and organic acids. The phenolics can be classified into two groups, the flavonoids and the non-flavonoids.

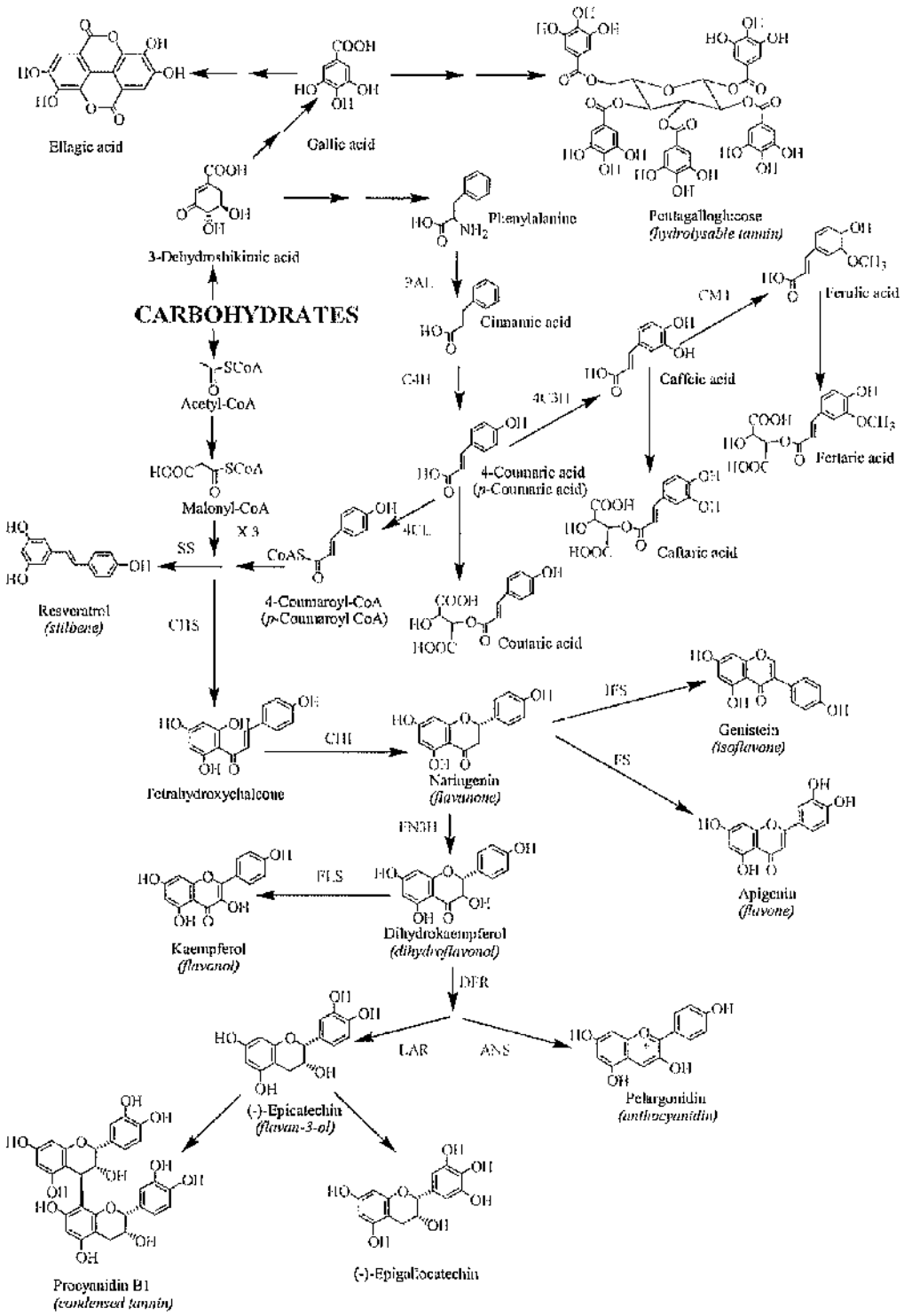


Figure 1.1. The Shikimate acid, phenyylpropanoid and flavonoid biosynthetic pathway
PAL, Phenylalanine ammonia-lyase; C4H, Cinnamate 4-hydroxylase; 4C3H, 4-Coumarate 3-hydroxylase; CMT, Caffeate methyl transferase; 4CL, 4-Coumarate: CoA ligase; SS, Stilbene synthase; CHS, Chalcone synthase; CHI Chalcone isomerase; IFS, Isoflavone synthase; FS, flavone synthase; FN3H, Flavanone 3-hydroxylse; FLS, Flavonol synthase; DFR, Dihydroflavonol 4-reductase; ANS, Anthocyanidin 4-reductase; LAR, Leucoanthocyanidin 4-reductase.

1.1.1. Flavonoids

Flavonoids are a group of polyphenolic secondary metabolites that occur naturally in most plants. They are found in large amount in fruits, vegetables, tea and wine. Flavonoids are sub-divided into several groups based on their degree of oxidation. The main subclasses are flavones, flavonols, flavan-3-ols, flavanones, isoflavones, and anthocyanins (Figure 1.2). All compounds present in each of these groups have the same basic structure (2-phenylbenzopyran). They have important and varied roles as secondary metabolites, including UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance (Koes *et al*, 1992).

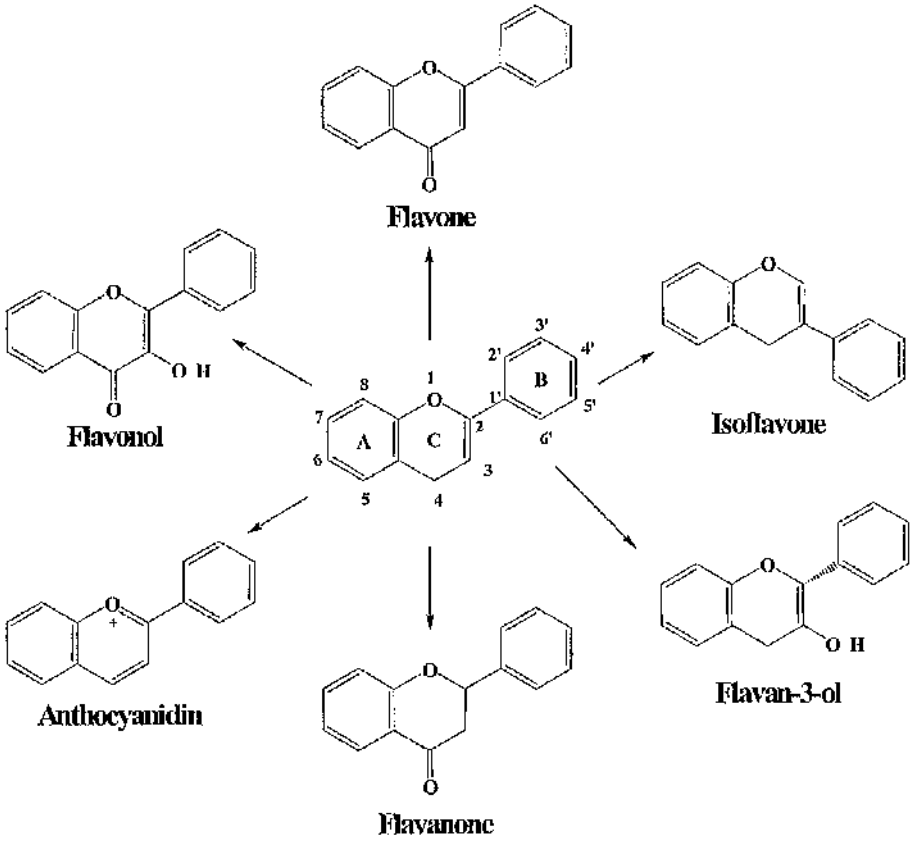
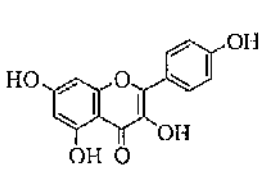


Figure 1.2. The generic structures of flavonoids

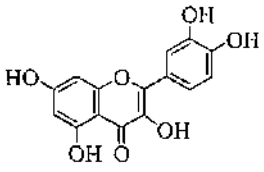
1.1.1.1. Flavonols

Flavonols are the most widespread of the flavonoids. The variability of flavonols is extensive, with over 380 flavonol glycosides and more than 200 different quercetin and kaempferol glycosides currently described (Bravo, 1998). The main flavonols are quercetin, kaempferol or myricetin and are usually found as *O*-glycosides (Figure 1.3).

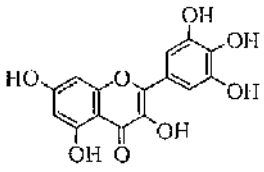
Conjugation occurs most frequently at the 3 position of the C-ring although substitutions at the 7, 4′, 3′ and 5′ also occur (Herrmann, 1976). The highest concentrations of these compounds occur in the leaves or fruits, where they act as UV protectants (Herrmann, 1976). There is extensive information concerning the level of flavonols in commonly consumed fruits and vegetables (Hertog *et al.*, 1992, 1993), where they are concentrated in the epidermis of leaves and skin of fruits. From a dietary perspective, levels will be low in skinned tomatoes and peeled apples.



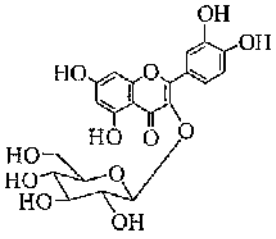
Kaempferol



Quercetin



Myricetin



Quercetin-3-*O*-glucoside

Figure 1.3. Structures of flavonols

1.1.1.2. Flavones

Flavones are structurally very similar to flavonols (see Figure 1.4). They differ from flavonols only in the absence of hydroxylation at the 3-position in the C-ring, however flavones are not so widely distributed (Crozier, 2003). Flavones are mainly found in celery (apigenin), sweet red pepper (luteolin), parsley and citrus fruits (polymethoxyflavones such as tangeritin). Chemically, flavones are also classified as 3-hydroxyflavones.

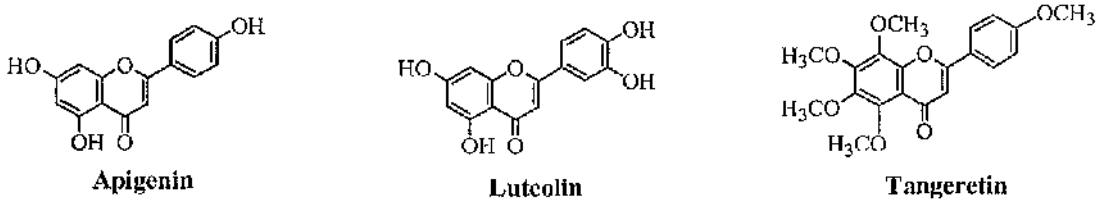


Figure 1.4. Structures of flavones and the polymethoxyflavone tangeritin

1.1.1.3. Flavan-3-ols

Flavan-3-ols (or flavanols) are a complex subclass of flavonoids ranging from the simple (+)-catechin and its isomer (-)-epicatechin, to the oligomeric and polymeric proanthocyanidins, also known as condensed tannins (see Figure 1.5).

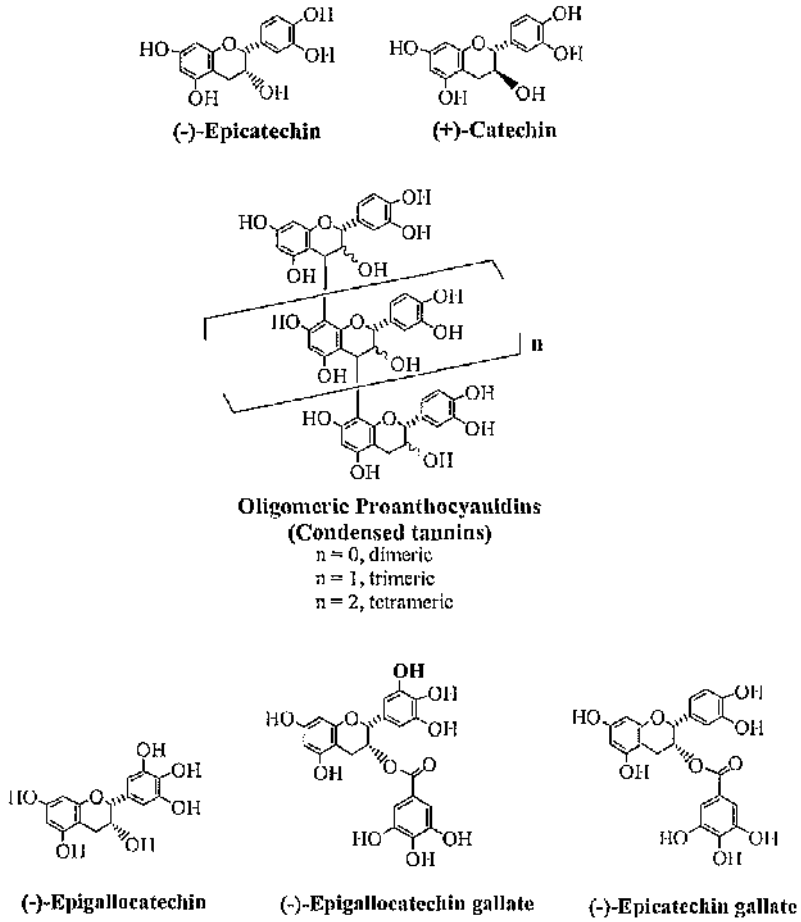


Figure 1.5. Structures of flavan-3-ols

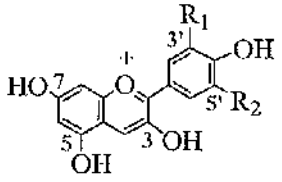
The size of proanthocyanidins could reach 50 polymers (Crozier, 2003). In addition to forming complexes with other flavan-3-ols, they also undergo esterification with gallic acid to form catechin gallates, and hydroxylation reactions to form

gallocatechins. Many of the flavan-3-ols are astringent and are involved in the taste and texture of food and confer a bitter and astringent taste to red wine or tea (Frankel *et al.*, 1993).

1.1.1.4. Anthocyanidins

Anthocyanidins are widely distributed throughout the plant kingdom, occurring in 27 families (Timberlake, 1988), particularly in fruits and flower tissues where they are responsible for the red, blue and purple colours. They play an important role in attracting insects to flowers for pollination but are also involved in protecting the plant against UV light (Crozier, 2003).

The most common anthocyanidins are malvidin, cyanidin, delphinidin, pelargonidin, petunidin and peonidin (cf Figure 1.6). These compounds are naturally found as their sugar-conjugated derivatives, called anthocyanins. But they can also be conjugated to hydroxycinnamates and organic acid such as malic acid and acetic acid. The conjugation occurs most often on carbon 3, although 5, 7, 3′ and 5′ substitution occurs.



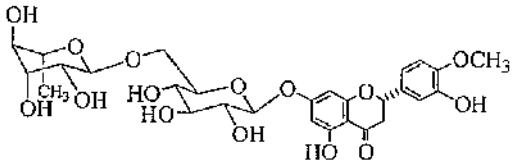
Anthocyanidin	R_1	R_2	Colour
Pelargonidin	H	H	orange-red
Cyanidin	OH	H	red
Delphinidin	OH	OH	pink
Peonidin	OCH_3	H	bluish purple
Petunidin	OCH_3	OH	purple
Malvidin	OCH_3	OCH_3	redish purple

Figure 1.6. Structures of anthocyanidins

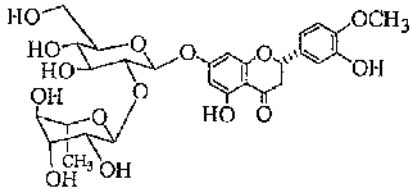
1.1.1.5. Flavanones

Two main structural features characterise the flavanones, the absence of the C2-C3 double bond, and the presence of a chiral centre at C2 (cf Figure 1.7). In naturally occurring flavanones, the C2 phenyl group is mainly orientated downwards in the

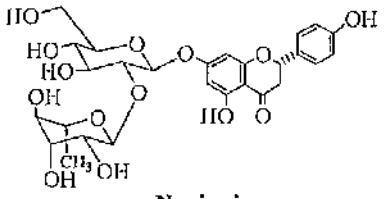
α-configuration. The flavanone structure is highly reactive and they have been reported to undergo hydroxylation, glycosylation and *O*-methylation reactions (Crozier, 2003). The most common flavanones are naringin and hesperidin. Flavanone rutinosides are tasteless but most of their other glycosides have an intensely bitter taste (Kanes *et al.*, 1993). They are found mainly in citrus fruits and juices.



Hesperidin
(hesperitin-7-*O*-rutinoside)



Neohesperidin
(hesperitin-7-*O*-neohesperidoside)



Naringin
(naringenin-7-*O*-neohesperidoside)

Figure 1.7. Structures of flavanones

1.1.1.6. Isoflavones

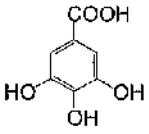
Structurally isoflavones differ from the common flavonoid in the orientation of the B ring. They are characterised by having the B ring attached at the C3 rather than the C2 position. Isoflavonoids undergo hydroxylation and methylation reactions. Isoflavonoids are best known for their oestrogenic activity as they seriously affect the reproduction of grazing animals. It has been suggested that they may have a role in the prevention of breast cancer and osteoporosis (Scalbert and Williamson, 2000). The best-known isoflavonoids are daidzein and genistein. Their principal source is legumes such as soybeans, black beans, green split peas and sprouts.

1.1.2. Non-flavonoids

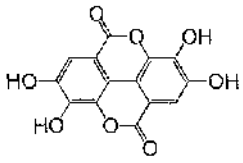
Non-flavonoids consist of one or two benzene rings and are derivates of benzoic acid, cinnamic acid and their respective aldehydes. They are subdivided into hydroxybenzoates, hydroxycinnamates and other minor families.

1.1.2.1. Hydroxybenzoates

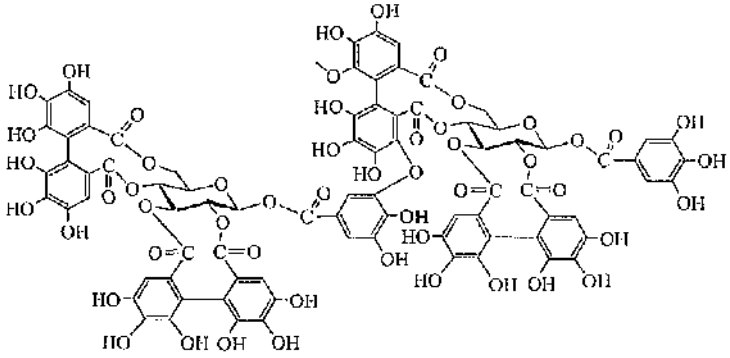
The hydroxybenzoates are derivatives of gallic acid and its dimer ellagic acid. Ellagic acid is the basic unit of the ellagitannins, whereas gallic acid is the main constituent of the gallotannins, both known as hydrolysable tannins (Figure 1.8).



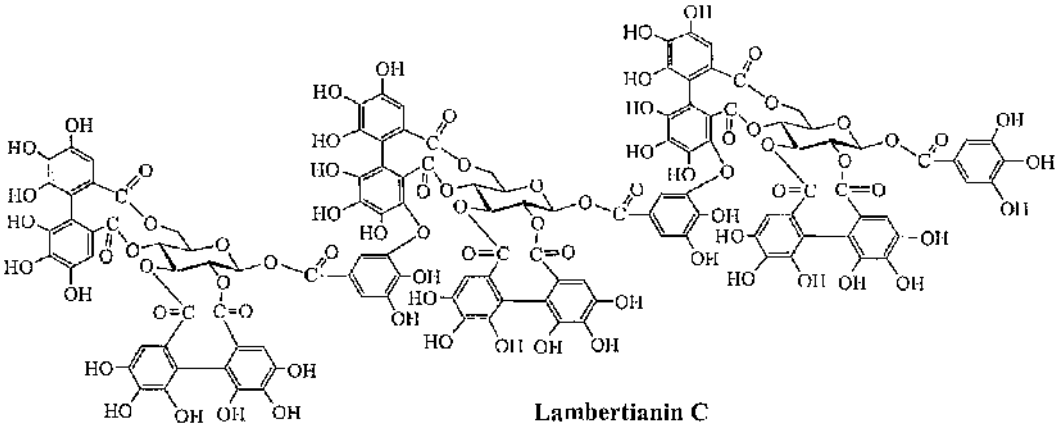
Gallic acid



Ellagic acid



Sanguiin H-6



Lambertianin C

Figure 1.8. Structure of gallic acid, ellagic acid and the ellagitannins sanguiin H-6 and Lambertianin C

1.1.2.2. Hydroxycinnamates (Figure 1.9)

Hydroxycinnamates are derivatives of cinnamic acid. The most common hydroxycinnamates are caffeic, *p*-coumaric, caffeic, ferulic and sinapic acids which are produced by a series of hydroxylation and methylation reactions. Chlorogenic acid (5-O-caffeoylquinic acid) or neochlorogenic acid (3-*O*-caffeoylquinic acid) are derivatives of caffeic acid and are common in food and beverages, such as lettuce and coffee (Ferreres *et al.*, 1997). Hydroxycinnamic acids are rarely found in their free form, tending instead to accumulate as glycosylate conjugates in most plant tissues including pollen (Harborne, 1993).

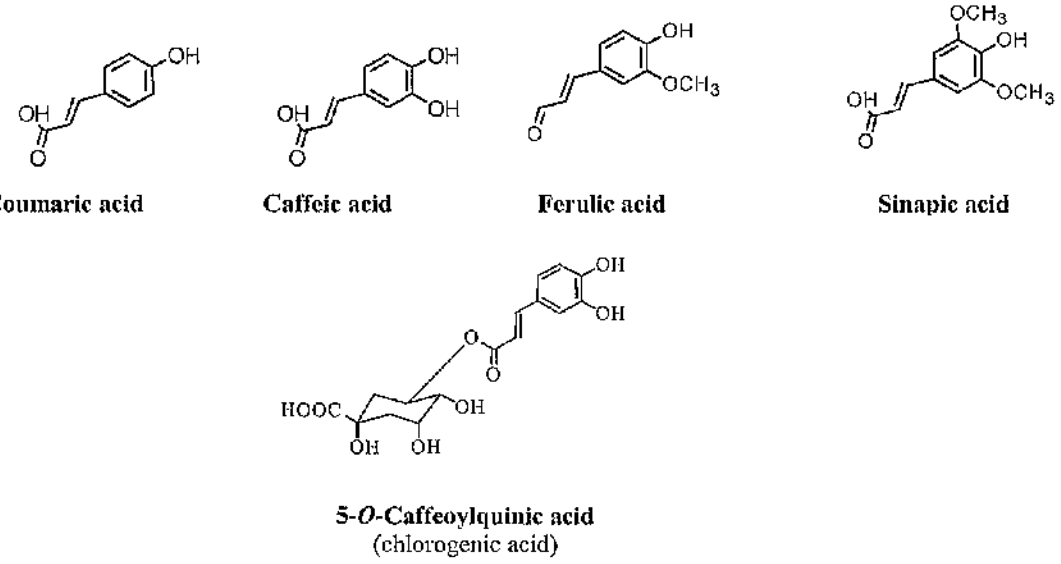


Figure 1.9. Structures of hydroxycinnamates

1.1.2.3. Stilbenes

Stilbenes are produced in plants as a consequence of pathogen infection, due to their ability to inhibit growth (Fremont, 2000). The best known compound is *trans*-resveratrol, which is found in red wine.

1.1.2.4. Other families

The remaining compounds which make-up the non-flavonoids are from other minor families such as acetophenones, coumarins, chalcones, xanthones and lignans.

1.2. Dietary source and intake of phenolics

Fruits and vegetables contain many phenolics. Factors such as variety, soil, climatic conditions, agricultural methods, degree of ripeness or storage conditions affect the content of bioactive compounds in individual fruits and vegetables (Crozier *et al.*, 1997). The level of phenolics also varies within the plant; within fruits many are concentrated in the skin, and within vegetables in the outer leaves. The content determined by analysis could therefore vary between studies by a factor of 20 to 100 fold (Price *et al.*, 1987). Furthermore there is a lack of information available on the levels of phenolic in foodstuffs. Additionally as diets are quite different between populations, estimating dietary intake of phenolics is quite difficult. Nonetheless, Hertog *et al.* (1993) estimated flavonoid consumption in the Netherlands to be 23 mg/day. This consumption was restricted to 3 flavonols and 2 flavones, it is therefore almost certainly a serious underestimate. At the other end of the scale Kuhnau (1976) proposed an intake of over 1000 mg/day in the USA, considerating all phenols being glycosides, which is likely to be an overestimate.

1.2.1. Beverages

Tea and wine provide the greatest proportion of beverage-derived phenolic intake. Red wine contains between 100 and 4000 mg/L phenolics, mainly derived from the anthocyanins and the proanthocyanidins (Burns, 2000). (+)-Catechin is one of the most abundant phenolic compounds in red wine, but phenolic acids, flavonols, monomeric and polymeric catechins are also present. In tea, the most abundant phenolics are catechins, the most prevalent of which is epigallocatechin gallate, and their polymers theaflavins and thearubigens, as well as some flavonols (Kris-Etherton *et al.*, 2002). Coffee and cocoa also contain high amount of phenolics. In coffee, the main phenolic is 5-*O*-caffeoylquinic acid, with higher levels detected in instant coffee (22989 – 23845 mg/kg) than ground roast coffee (15545 – 19176 mg/kg) (Lakenbrink *et al.*, 2000). Cocoa contains levels of phenolics comparable to those of ground roast coffee. Phenolics present include catechins, procyanidins and anthocyanins as well as quercetin and quercetin-3-glucoside (Kris-Etherton *et al.*, 2002).

1.2.2. Berries

Increased consumption of berries was one of the associated factors that resulted in a decline in CHD in Finland (Knekt *et al.*, 1996). Berries contain anthocyanins and a varying range of other flavonoids. Each berry has a different anthocyanin profile. While blackcurrants contain primarily delphinidin conjugates (1150-2500 mg/kg), raspberries and redcurrants contain cyanidin conjugates (100-600 mg/kg and 177 mg/kg respectively), pelargonidin conjugates predominate in strawberries at around 150-350 mg/kg (Froytlog *et al.*, 1998; Clifford, 2000; Maatta *et al.*, 2001). Many berries have been reported to contain high levels of flavonols, eg. lingonberries (169 mg/kg) or blackcurrants with 157 mg/kg (Hakkinen *et al.*, 2000). Berries are reputed to contain ellagic acid and are advertised on this basis in USA. However, raspberries contain very little ellagic acid and it only appears in high concentrations after extracts of the berries have been subjected to acid treatment, which results in the hydrolysis of the ellagitannins sanguiin H-6 and lambertianin (Mullen *et al.*, 2002a).

1.2.3. Citrus fruits

Citrus fruits are significant sources of flavonoids, principally flavanones and their glycosides conjugates, which are present in both the juice and tissues (Saltmarsh *et al.*, 2003). Citrus species also contain a number of polymethoxylated flavones, such as nobiletin or tangeritin, which are not commonly found in other plants (Manthey and Grohmann, 1996). They contain also significant amounts of terpenoids such as (+)-limonene in lemon and orange oils.

1.2.4. Tree fruits

Fruits are reported to contain high levels of flavonol glycosides and anthocyanins within their skin. In many fruits the red colour is due to anthocyanins.

Apples in particular are a good source of flavonoids (Podsedek *et al.*, 1998). Between 2310 and 4880 mg/kg have been determined in macerated apples including chlorogenic acid and its isomers, caffeic acid, phloridzin, (-)-epicatechin and its oligomers.

Plums are a rich source of anthocyanins, mainly present as cyanidin-3-glucoside or cyanidin-3-rutinoside (Saltmarsh *et al.*, 2003). In addition they have been reported to

contain significant quantities of chlorogenic acid, neochlorogenic acid and procyanidins (Tomas-Barberan *et al.*, 2001).

1.2.5. Vegetables

1.2.5.1. Root crops (e.g. carrots, turnips, ...)

In root crops, the principal compounds of interest are α and β-carotenes. For example carrots contain up to 650 mg/kg (van der Berg *et al.*, 2000).

1.2.5.2. Onions and leeks

According to Hertog *et al.* (1993), over 48% of the daily total flavonol intake is from onions. Onions principally contain flavonol glycosides with on average 956 mg/kg quercetin-3,4′-diglucoside and 340 mg/kg of quercetin-4′-glucoside in yellow onions (Aziz *et al.*, 1998). Red onions additionally contain up to 250 mg/kg of anthocyanins (Clifford, 2000). By contrast, leeks have been found to have only 10-60 mg/kg of kaempferol conjugates and no quercetin.

1.2.5.3. Cabbage family and greens

All members of the genus Brassica contain glucosinolates, which are released in the presence of the enzyme myrosinase active compounds such as nitriles, thyocyanates and indole derivatives. In addition to glucosinolates, some brassicas contain flavonol conjugates and hydroxycinnamates. Broccoli florets have been reported to contain 65 mg/kg quercetin-3-sophoroside, 166 mg/kg kaempferol-3-sophoroside and 336 mg/kg sinapic acid conjugates (Plumb *et al.*, 1997). Spinach additionally contains axillarin-4-glucoside, spinacetin-3-gentobiose and other methoxyflavonol derivatives as well as conjugates of 4-coumaric acid (300-350 mg/kg) and a high level of carotenoids (Saltmarsh *et al.*, 2003).

1.2.5.4. Salad vegetables

In leafy vegetables flavonols are widely found. Lettuce in particular was reported to contain between 2 and 900 mg/kg quercetin for Iceberg and Lollo Rosso varieties

(Crozier *et al.*, 1997). Lettuce also contains caftaric and chlorogenic acids as well as carotenoids (Clifford, 1999).

1.2.5.5 Tomatoes and pepper

Tomatoes also occur widely in the diet. They contain flavonol glycosides mainly as quercetin-3-rutinoside in concentrations 1.3-22 mg/kg depending upon variety and growing conditions species (Stewart *et al.*, 2000), carotenoids (mainly as lycopene), chlorogenic acids as well as the flavanone naringenin (Paganga *et al.*, 1999).
Peppers contain several carotenoids, mainly lutein or β-carotene. The different colours are due to the levels of carotenoids: yellow peppers accumulate lutein whereas capsanthin is responsible of the red bell colour (Saltmarsh *et al.*, 2003).

1.3. Function of phenolics in plants

Even if they have no primary role in plant function, phenolics are essential to the plant's physiology as they have been implicated in stress defence including UV protection, disease resistance and pigmentation as described by Koes *et al.*, 1994.

1.3.1. UV protection

If plants need UV exposure to do photosynthesis, this can cause damage to DNA. The accumulation of phenolic compounds in the upper epidermal tissues - in particular the flavonols and hydroxycinnamates, which are absorb maximally in the UV region of 280 to 340 nm - contribute to preventing of UV radiation induced tissue damage (De Lucia *et al.*, 1992).

1.3.2. Disease defence

Some phenolic compounds have been ascribed a role in plant defence against microbial attack (viruses, bacteria, pathogenic fungi…). In a defensive response, plants produce a range of compounds called phytoalexins, which are toxic to the attacking pathogen. For example the stilbene *trans*-resveratrol is known to be produced in vine stems, leaves and grape tissue in response to attack by *Botrytis cinerea*.

1.3.3. Pigmentation

The most important phenolic pigments are the flavonoids, in particular the red/purple anthocyanins, and the yellow aurones and chalcones. Pigmentation has an important role to play in the attraction of pollinating birds and insects. Furthermore, pigmentation may encourage the consumption and subsequent distribution of seeds (Crozier, 2003).

1.3.4. Others

Phenolics have also been reported to act as signal molecules in the formation of nitrogen-fixing root nodules in legumes. They are also plant hormone secretion controllers and enzyme action inhibitors (Markham, 1989). Furthermore, flavonoids produce stimuli to assist in pollination and are involved in the regulation of pollen tube growth in the stigma, and are able to produce stimuli to guide insects to their food source (Cook & Samman, 1996).

1.4. Health benefits of Phenolic compounds

Flavonoids were initially named vitamin P and their protective effect against human diseases was identified before the active ingredients had been isolated (Rusnyak, 1936). Flavonoids are no longer described as vitamins, as they are not essential for human health but their presence in the diet can have many beneficial effects, in part due to their antioxidant properties.

1.4.1. Antioxidant properties of phenolics

Natural metabolism in plant, animals and human produces reactive oxygen species such as the hydroxyl ($OH^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$) and the superoxide anion ($O_2^{\bullet-}$). These free radicals contain an unpaired electron and could be highly reactive, generating further radicals. They can react with and damage DNA, proteins and lipids and they have been implicated in diseases such as atheriosclerosis, various cancers, chronic inflammatory diseases and Parkinson's disease (Wang *et al.*, 1999).

As well as the common antioxidants such as vitamin C and E, polyphenols appear to be involved in antioxidant defence. Indeed polyphenolic compounds have been shown to possess antioxidant activity by merit of their structure, particularly the number and orientation of their hydroxyl groups. Consumption of phenolic-containing food and beverages results in an increase in antioxidant activity of plasma (Nakayama, 1994). Polyphenols could act at different levels (Rice Evans *et al.*, 1997); they can directly scavenge free radicals or act as "secondary" antioxidants. Rather than scavenging free radicals themselves they act to regenerate vitamins E or C when they accept/donate an unpaired electron or hydrogen from a free radical.

1.4.2. Anti-carcinogenic activity

Many studies have demonstrated an inhibition of cancer activation by tea, red wine or fruits and vegetable polyphenols *in vitro* (Ebeler *et al.*, 1997; Yang *et al.*, 2000), such activities have been observed in only a few *in vivo* studies and results are sometimes contradictory. Indeed, in a Finnish cohort study with 9,959 people, Knekt *et al.* (1997) observed an inverse association between the intake of flavonoids and the incidence of all sites of cancer combined. This association was mainly a result of lung cancer prevention. The Zuthphen elderly study (Hertog *et al.*, 1994) conducted with 738 men reported that flavonoid intake was not associated with incidence of all-case cancer. Nonetheless, high intake of flavonoids from fruits and vegetables was inversely associated with risk of cancer of the alimentary and respiratory tract. In Finland the α-Tocopherol, β-Carotene Cancer Prevention (ATBC) study (Hirvonen *et al.*, 2001) with 27,110 male smokers reported the intake of flavonols and flavones to be inversely associated with the risk of lung cancer, but not with that of other cancers (urothelial, renal, prostate, colorectal or stomach). Garcia-Closas *et al.* (1998) in a case control study including 354 cases of gastric cancer and 324 controls reported quercetin and kaempferol to be protective against gastric cancer, whereas no similar association was found for carotenoids. More recently a case control study in Greece (Peterson *et al.*, 2003) showed an inverse association of breast cancer and consumption of vegetables. These authors took their study a step further to try and see which flavonoids in particular were responsible for the protective effect by controlling the intake of fruits and vegetables and other flavonoids. They reported a strong significant inverse association of flavone intake with breast cancer, whereas this association became non

significant with flavanones, flavan-3-ols, flavonols, anthocyanidins or isoflavones. Further investigations are required to clarify the situation and define more precisely which compounds are responsible for the protective effects.

1.4.3. Anti-atherogenic activity

In France, especially in the south-west, death rates from coronary heart disease are very low, despite the consumption of diets high in fat. This phenomenon is known as the "French paradox" (Renaud *et al.*, 1992). This was assumed to be due to the high consumption of red wine, with the active ingredients being phenolic and polyphenolic components. Epidemiological studies are not so clear. In a cohort study in Finland (Knekt *et al.*, 1996) coronary mortality was significantly inversely associated with flavonoid intake for women, but in men the inverse association was not significant. Attention has been drawn to tea as a potential cardioprotective beverage (Hertog *et al.*, 1993; Geleijnse *et al.*, 2002). Nonetheless, a study by Sesso *et al.* (2003) did not support an association between tea intake and coronary or cardiovascular disease in a prospective study including 17,228 subjects.

Artherosclerosis is due to a combination of hyperlipidemia and oxidation of lipoproteins. Low density lipoproteins (LDL), a group of plasma lipoproteins, which transport cholesterol to peripheral issues and regulate cholesterol synthesis are involved in the formation of atherosclerosis. They are internalised, trapped and begin to accumulate in the vessel cell wall. At this stage, free radicals oxidise LDL through lipid peroxidation. Subsequently, oxidation of LDL induces modification in lipoproteins, stimulates inflammatory reactions, causes monocytes and monocyte-derived macrophages to accumulate large amounts of oxidised LDL, forming lipid-laden foam cells and atherosclerotic plaques by merging. This leads to the formation of a fatty streak and vascular occlusion causing coronary heart disases (CHD). The antioxidant activity of phenolics suggests they can scavenge the free radicals, which would otherwise cause the oxidation of LDL (Puddey and Croft, 1997; Fuhrmann *et al*, 2001; Frankel *et al*, 1993).

1.4.3. Anti-inflammatory activity

Phenolics have been suggested to increase the production of anti-inflammatory cytokines by human leukocytes *in vitro* (Crouvezier *et al.*, 2001). The anti-inflammatory action of phenolics could then explain their ability to reduce susceptibility to conditions such as Alzheimer diseases.

1.4.4. Anti-viral and bacterial activity

Phenolic compounds are believed to bind to viral proteins, therefore affecting synthesis of viral nucleic acids. Several studies have shown that consumption of cranberry juice can reduce the risk of urinary tract infection (Kontiokari *et al.*, 2001). The phenolics are believed to inhibit the adhesion of the infecting bacteria (*Escherichia Coli*) to the uro-epithelial cells. (Howell *et al.*, 1998).

1.4.5. Vascular function

Even as early as 1936 it was realised that flavonols induced effects on the human vascular system. Extracts of red pepper were known to decrease the permeability of the capillary walls (Rusznyak *et al.*, 1936). There is some evidence that grape juice and wine polyphenols in particular can increase the production of nitric oxide by the vascular endothelium, resulting in vasorelaxation (Kris-Etherton *et al.*, 2002). This can help to reduce the risk of CHD, hypertension or diabetes.

1.4.6. Skin Chemoprotection

Cyclobutane pyrimidine dimers form in skin cells, following exposure to excessive levels of UV resulting in damage to DNA, which can lead to skin cancer. Green tea polyphenolics have been shown to reduce the incidence of skin cancer of hairless mice (Wang *et al.*, 1994).

1.4.7. Oestrogenic activity

Isoflavones such as genistein have some oestrogenic activity due to the similarity in the structure. Countries in which the dietary intake of such isoflavones is high (due to the

inclusion of soya), the occurrence of osteoporosis, menopausal symptoms and even breast or prostate cancer are relatively low (Cassidy *et al.*, 2000).

1.5. Absorption and metabolism of phenolics

Data concerning absorption and bioavailability of phenolic compounds is essential before biological activity and nutritional potential value can be estimated. All flavonoids except flavan-3-ols are present in plants bound to different sugars as glycosides. The nature of the sugar may also affect the absorption of flavonoids. The available literature mainly concerns the absorption of flavonols - quercetin in particular, anthocyanins, and more recently flavan-3-ols, whereas few studies have investigated the biovailability of flavanones, flavones and other polyphenols. With the increasing use of analytical procedures such as HPLC-tandem mass spectrometry (MS-MS), recent investigations have begun to build up a picture of how, following ingestion, dietary flavonoids are metabolised in the body, principally to glucuronide, sulphated and methylated derivatives. The route of absorption and metabolism is shown in Figure 1.10.

1.5.1. Flavonols

Initial work on the absorption of flavonol compounds was contradictory. Absorption of flavonols was first thought to be dependant on whether they were present as flavonol glycosides or aglycones. In fact absorption of conjugated flavonols was suggested to be delayed until the large intestine, where enzymes could act on these compounds to produce aglycones, as the presence of a hydrophobic sugar residue was believed to prevent them to crossing the membrane of the small intestine (Kuhnau, 1976). Now there is evidence to suggest that some flavonol conjugates are preferentially absorbed in comparison to aglycones (Hollman *et al.*, 1997; Aziz *et al.*, 1998). It was suggested that quercetin glycosides could interact with glucose transporters (SGLT1) in the small intestine and so be absorbed into the blood system (Gee *et al.*, 1998).

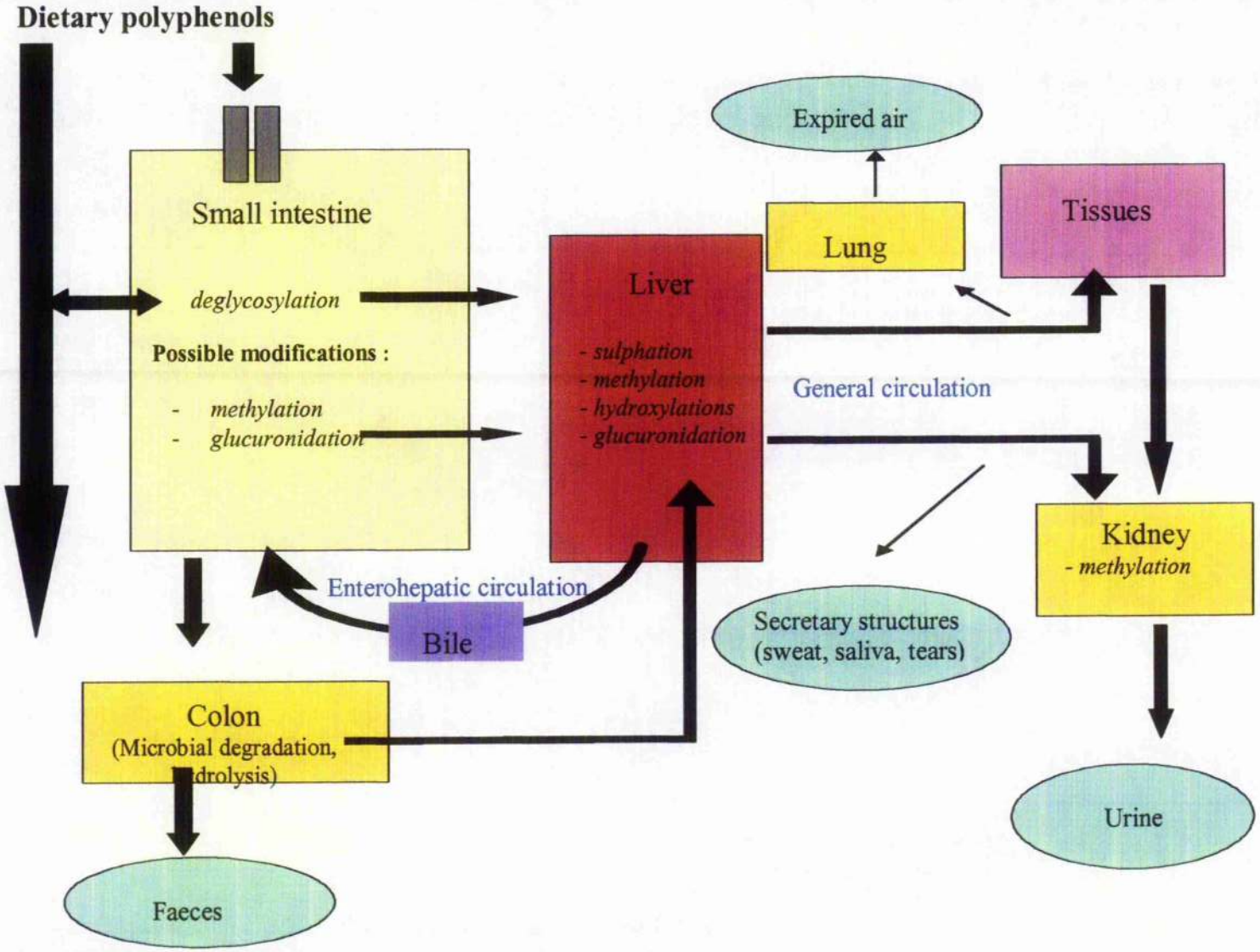


Figure 1.10. Possible route for dietary polyphenols metabolism

More recently, Day *et al.* (2001) detected a mixture of 12 putative glucuronidated and sulphated conjugates of quercetin and methylquercetin in human plasma after the ingestion of fried onions. There was therefore, extensive metabolism of the parent flavonol glucosides involving deglycosylation, glucuronidation, sulphation and methylation. It was suggested quercetin glucosides were deglycosylated by β-glucosidases (cytosilic-β-glucosidase and lactase phloridzin hydrolase) in the small intestine. The aglycones does not accumulate but was metabolised by uridine-5'-diphosphate glucuronyl-transferase, sulphotransferases and/or catechol-*O*-methyltransferases (O'Leary *et al.*, 2003). Quercetin metabolites could then reach the liver, where they may be further methylated, glucuronidated or sulphated. Quercetin-3-*O*-rutinoside is not a substrate for β-glucosidases and appears not to be absorbed in the small intestine but travels to the large intestine, where it is probably degraded by colonic bacteria (Nemeth *et al.*, 2003).

1.5.2. Flavan-3-ols

Interest in the bioavailability of flavanols has increased due to their occurrence in red wine, tea and chocolate. Piskula and Terao (1998) reported absorption of (-)-epicatechin in rats was taking place before glucuronidation in the intestinal mucosa. They hypothesised that the conjugated form of (-)-epicatechin entered the portal vein, and was sulphated in the liver, methylated in liver or kidney, before being excreted in bile or urine. In humans (Donovan *et al.*, 1999; Bell *et al.*, 2000) different catechin metabolites (mainly catechin sulphate and/or glucuronide conjugates as well as lower level of free catechin and methylcatechin) were excreted from the body after consumption of a glass of wine. Methylated metabolites were eliminated quicker than unmethylated ones. However the overall levels identified were low compared to catechin levels of the wine itself. More recently Gonthier *et al.* (2003a and b) reported that in rats fed a catechin-rich diet, microbial aromatic acid metabolites were formed in the gut and excreted in urine. The main ones excreted were 3-hydroxyphenylpropionic acid and to a lesser extent 3-hydroxybenzoic acid and 3-hydroxyhippuric acid, as well as traces of 4-hydroxybenzoic acid, 3,4-dihydroxyphenylpropionic acid, 3,4-dihydroxyphenylacetic acid and ferulic acid. It seems the degree of procyanidin polymerization has a major impact on the fate of metabolites as absorption through the

gut barrier is poor and metabolism by the intestinal microflora is limited as compared to catechin (Gonthier *et al.*, 2003b).

Concerning the absorption and metabolism of proanthocyanidins in rats or human, very little is known as the limited availability of pure standards has made feeding studies difficult. Nonetheless Deprez *et al.* (2000) reported that procyanidins (average polymerisation of 6) are degraded after 48 h incubation with human colonic microflora into low-molecular-weight aromatic acids such as monohydroxylated, phenylacetic, phenylpropionic and phenylvaleric acids. Holt *et al.* (2002) detected the presence of dimeric procyanidins B2 in human plasma as early as 30 minutes after the consumption of cocoa (containing monomer to decamer of catechin).

1.5.3. Anthocyanins

Absorption and excretion of anthocyanins differ from that of other phenolics as they appear to be mainly excreted unmetabolized in the glycosylated form in which they were administrated, but in very low concentrations, usually 0.1% or less, of the ingested dose (McGhie *et al.*, 2003). Some authors describe limited absorption of anthocyanins from blackcurrants (Nielsen *et al.*, 2003; Matsumoto *et al.*, 2001) and elderberries (Cao *et al.*, 2001) in humans. Anthocyanins are rapidly absorbed into the blood and excreted within two hours of ingestion as intact forms. Thus, glycosylated anthocyanins, unlike flavonol glycosides, are absorbed from the gastrointestinal tract and appear in the blood stream. This may be a consequence of the fact that, in contrast to quercetin glucosides, anthocyanin glucosides are not hydrolysed by human small intestine β-glucosidases (Nemeth *et al.*, 2003). It seems than the anthocyanin rutinosides are more readily absorbed than the corresponding glucosides (Nielsen *et al.*, 2003). Miyazawa *et al.* (1999) suggest a dose-dependent level of absorption with the amount of cyanidin glucosides increasing significantly in plasma with increasing amount of anthocyanin ingested.

Other studies (Tsuda *et al.*, 1999; Miyazawa *et al.*, 1999) analysed the distribution of anthocyanins metabolites in plasma and urine of both human and rats as well as in liver, kidney, stomach and small intestine of rats after the ingestion of pure cyanidin-3-glucoside. Cyanidin-3-glucoside was identified unchanged in plasma,

whereas methylated cyanidin-3-glucoside was detected in both the liver and kidneys. No aglycone, glucuronide or sulphate metabolites were detected in these studies.

Wu *et al.* (2002) fed elderberries to humans and by using improved analytical techniques, found, in addition to the unmetabolised forms (cyanidin-3-glucoside and cyanidin-3-sambubioside), four metabolites, peonidin-3-*O*-glucoside, peonidin-3-*O*-sambubioside, a peonidin glucuronide and a cyanidin-3-*O*-glucosylglucuronide in urine. This demonstrates that methylation of the 3′-hydroxyl group had occurred as well as glucuronidation. In a study with strawberry, which contain pelargonidin-3-glucoside, the predominant anthocyanin to appear was this time not the parent glucoside but three pelargonidin sulphate and the aglycone pelargonidin. (Felgines *et al.*, 2003).

1.6. Phenolics in redcurrants

There are several reports on the phenolic content of a range of berries including raspberries, blueberries, or blackcurrants. However the phenolic content of redcurrants has been investigated rarely and the available data are very contradictory. Although anthocyanins were analysed directly, all other phenolics were analysed after acid hydrolysis of extracts, which results in sugar conjugates being cleaved releasing the aglycones.

1.6.1. Anthocyanins

Anthocyanins are responsible for the colouring of redcurrants. In redcurrants, cyanidin is the only aglycone present (Oydin, 1973). Five different glycosides of cyanidin in redcurrant were found (Maatta *et al.*, 2001, Goiffon *et al.*, 1999) including mainly cyanidin-3-(2-glucosyl)-rutinoside and cyanidin 3-(2-xylosyl)-rutinoside as well as cyanidin-3-rutinoside, cyanidin-3-glucoside and cyanidin-3-sophoroside. The total content of anthocyanins in redcurrants varies from 177 mg/kg fresh weight (Maata *et al.*, 2001) to 1130 mg/kg dry weight (Kahkonen *et al.*, 2001).

1.6.2. Flavonols

Data on flavonols are more contradictory. In most studies, no free flavonol aglycones were detected, and redcurrants were acid hydrolysed prior to analyses. With the exception of Lugasi *et al.* (2002), quercetin was detected after acid hydrolysis in quantities ranging from 8 mg/kg (Justesen *et al.*, 1998) to 20.6 mg/kg of fresh fruit (Amakura *et al.*, 2000). Some studies did not detect myricetin and kampferol in acid hydrolysed samples (Justesen *et al.*, 1998, Hertog *et al.*, 1992, Hakinen *et al.*, 1999), whereas Amakura *et al.* (1999) report the presence of 3.7 mg/kg myricetin and 20.6 mg/kg of kaempferol. Lugasi *et al.* (2002) detected myricetin (42.9 mg/kg) but no kaempferol.

1.6.3. Flavan-3-ols and procyanidins

Information from early literature on the levels of flavan-3-ols was limited. By spectral identification, Kahkonen *et al.* (2001) found 680 mg/kg dried redcurrants of flavanols (expressed as catechin equivalents). This corresponds to approximately 68 mg/kg fresh weight. Maatta *et al.* (2001) were able to identify (+)-catechin by comparison of ultraviolet spectra but it was not possible to quantify flavan-3-ols. Concerning the proanthocyanidins, non-extractable proanthocyanidins were quantified in delphinidin equivalents, as upon acid hydrolysis the residue release anthocyanidin pigments. Redcurrants were found to contain 34 mg/kg fresh weight proanthocyanidins, expressed in delphinidin equivalents.

1.6.4. Hydroxybenzoates

Hydroxybenzoic acids have also been found in redcurrants. Kahkonen *et al.* (2001) reported finding 140 ± 10 mg/kg in dried berries; Hakkinen *et al.* (1999) detected a similar range of values (15.9 mg/kg fresh redcurrants). Maata *et al.* (2001) found 4-hydroxybenzoic acid (3 mg/kg) and dihydroxy-(methoxy)-benzoic-acid (3 mg/kg). Schuster *et al.* (1985) reported 7-13 mg/kg fresh redcurrants of 4-*p*-hydroxybenzoic acid-glucoside.

1.6.5. Hydroxycinnamates

Hydroxycinnamic acid derivatives were detected in some of the studies. Hakkinen *et al.* (1999) claimed to find 94 mg/kg in dried redcurrants, but provided no details on the nature of the acids involved. Shuster *et al.* (1985) were looking specifically at the content of quinic acid and glucose esters of hydroxycinnamic acids by capillary GC and HPLC in soft fruits including redcurrants. They reported the presence of caffeoylquinic acid, *p*-coumaroylquinic acid and traces of feruloylquinic acid as well as glucose ester of caffeic acid (2-5 mg/kg fresh weight), *p*-coumaric acid (2 mg/kg) and ferulic acid (traces), and glucoside forms with 2 mg/kg caffeic acid glucoside and 5-16 mg/kg *p*-coumaric glucoside. More recently, Maata *et al.* (2001) reported finding two major non-polar peaks with spectral characteristics similar to those of *p*-coumaric acid and caffeic/ferulic acids after acid hydrolysis. The levels detected were quite low (3 mg/kg for *p*-coumaric acid and 5mg/kg for caffeic/ferulic acid).

1.7. Phenolics in organic and non-organic fruits and vegetables

There is growing consumer interest in organic produce, which anecdotally are thought to be healthier than conventionally-produced agricultural crops. Flavonoids and other phenolic compounds are produced by plants in response to viral and microbial infection and stress in the plants (Koes *et al.*, 1994). As no pesticides are used in the production of organic crops, it is feasible that they contain elevated levels of phenolic as compared to the conventionally grown equivalents (Asami *et al.*, 2003). There is however, a lack of scientific evidence to support this proposition. Most studies have tended to focus on a narrow range of nutrients, which give only a very limited indication of nutritional values. Defining organic and conventional crops is already quite difficult. Organic products are produced under controlled cultivation conditions; referring to EC regulation in UK, they are grown without using chemical synthetic pesticides and herbicides and cannot be genetically modified or irradiated. However using crop rotation systems and extensive soil tillage, conditions may exist, which are not specific for organic crops. Conventional agriculture evolved in response to technological mechanisation/tillage, monoculture, synthetic fertilizers, irrigation, chemical pest and weed controls, and genetic advances in breeding. Moreover as other factors, such as

variety, stage of maturity, storage, light exposure or type of soils affect the levels of phenolic compounds, it is very difficult to compare directly organic production practices to conventional ones (Felsot and Rosen, 2004). This could explain why papers are to date contradictory. A recent review summaring the results of around 150 of these studies (Woese *et al.*, 1997) found inconsistencies in nutrient content of conventionally and organically produced crops, except for the nitrate content which tends to be lower in organically grown fruits and vegetables. As far as the vitamin C content is concerned no major difference was observed in half of the studies, while the other half revealed a trend towards a slightly higher content in organically cultivated fruits and vegetables (Woese *et al.*, 1997). Weibel *et al.* (1999) found a higher content of phenolic compounds in organic apples compared to conventional ones but no consistent differences were reported between organic and conventionally cultivated strawberries (Hakkinen *et al.*, 2000). In another study (Asami *et al.*, 2003), the results demonstrate a significant trend towards higher levels of total phenolic content in organically produced crops (marionberries, strawberries and corn, same variety, same farm and harvested at the same time to ensure the same degree of ripeness) compared to conventionally produced crops. More recently Lombardi-Boccia *et al.* (2004) reported significantly higher phenolic acid content in conventional plums compared to organic fruit, whereas the β-carotene concentration in organically grown plums was higher than that found in conventionally grown plums and no differences were established in ascorbic acid levels.

Fewer reports are available concerning the impact of organic or non organic diets on humans. Grinder-Penderson *et al.* (2003) compared conventional and organic produced diets in a human crossover intervention study. The organic diet contained significantly increased amounts of quercetin and kaempferol compared to conventional diet, whereas there was a trend toward a higher content of isorhamnetin in the conventional diet. The excretion of flavonoids in urine was higher after 22 days of intake of the organically produced diets compared to the conventional one. There was no difference in the level of biomarkers of antioxidative status (activity of superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase in erythrocytes and ferric reducing ability of plasma) between the two diets except for the Trolox equivalent antioxidant capacity (TEAC) measurement, where the values were significantly higher after intake of the conventional diet.

1.8. Aims of the study

Recent evidence has suggested that diets rich in fruits and vegetables may protect against coronary heart disease and certain cancers. Fruits and vegetables are a rich source of antioxidants, including phenolic and polyphenolic compounds. The nature and content of polyphenols vary from plant to plant and the contribution of individual components is not yet well known. There is also growing interest in organic products, which anecdotally are thought to be healthier than conventional produce, although at present there is a lack of scientific evidence to reinforce this claim.
This study set out to achieve the following aims:

1. Identify and quantify the major phenolic compounds contributing to the *in-vitro* antioxidant capacity of a range of fruits and vegetables.
2. Compare antioxidant capacity, phenolic content and vitamin C levels in organic and conventional grown vegetables obtained from local stores.
3. Investigate the absorption and excretion of anthocyanins, using human volunteers with an ileostomy.

CHAPTER 2. MATERIALS AND METHODS 29

Chapter 2. Materials and Methods

2.1. Collection and extraction of fruits and vegetables analysed

2.1.1. Organic and conventional fruits and vegetables

All organic and conventionally fruits and vegetables were purchased from Safeway Stores, Sainsburys Plc and Roots & Fruits in Glasgow depending on their availability in the market. Details of origin and date of collection are shown in Table 2.1. Whenever possible, comparable products from the same country of origin were chosen. Samples were collected, immediately frozen in liquid nitrogen, lyophilised, powdered and stored at -20°C prior to analysis.

Table 2.1. Organic and conventional fruits and vegetables analysed

		Organic		Conventional	
Product	***Date***	***Origin***	***% water***	***Origin***	***% water***
Carrot	02/09/02	Austria	90.5	Austria	90.5
Apple	02/09/02	Italy	85.9	Italy	85.7
Orange	02/09/02	New Zealand	73.6	Spain	83.9
Kiwi 1	02/09/02	Chile	86.1	Chile	83.1
Kiwi 2	29/01/03	Italy	89.6	Italy	89.4
Broccoli 1	08/08/02	United Kingdom	90.7	United Kingdom	89.5
Broccoli2	29/01/03	Italy	86.1	Italy	77.4
Cauliflower	06/01/03	United Kingdom	91.7	United Kingdom	91.5
Potato	06/01/03	Scotland	87.0	Scotland	81.3
Sweet Potato	21/01/03	Israel	83.74	Israel	84.4
Banana	06/01/03	Caribean Republic	75.5	Windward Isles	73.8
Pear	06/01/03	Italy	86.8	England	85.9
Green bean	21/01/03	Egypt	91.2	Gambia	93.2
Cucumber	21/01/03	Spain	95.2	Spain	95.4
Onion	21/01/03	Holland	87.6	United Kingdom	90.4
Red pepper	21/01/03	Israel	90.6	Israel	89.5
Beet root (cooked)	21/01/03	United Kingdom	85.6	United Kingdom	88.0
Cherry wine tomato	29/01/03	Spain	84.4	Spain	80.7
Garlic 1	02/09/02	Spain	62.3	United Kingdom	64.2
Garlic 2	29/01/03	Spain	61.3	Spain	60.0

Lyophilised tissues were weighted, placed on ice and homogenised in 4 mL methanol/water (1:1, v/v) using an Ultra-Turrax T25 homogeniser (IKA Werke, Staufen, Germany). The mixture was then centrifuged at 3000 g at 4°C for 5 minutes. The supernatant was stored at –80°C prior analysis.

2.1.2. Fruits, berries and vegetables for on-line antioxidant analysis

Fresh fruits and vegetables were purchased from local shops in Byres Road, Glasgow. Seven varieties of berries (blueberries, cranberries, blackberries, strawberries, raspberries, blackcurrants, redcurrants and a range of fruits and vegetables (curly kale, kiwi, clementine, cherry tomato, purple broccoli, broccoli, red, yellow and green peppers) were selected on the basis of their high antioxidant capacity that was detemined in a previous study (Borges *et al.*, unpublished).

Phenolic compounds were extracted from fresh ground fruit and vegetable samples. Samples of fresh fruit and vegetable were ground with acidified methanol (0.1 % HCl) in a Waring blender. Aliquots were then extracted with 20 mL of acidified methanol, using an Ultratorrex for 1 min and centrifuged for 20 min at 4000 g. The pellets were extracted 3 more times; supernatants were mixed together, then concentrated using a rotovapor, and resuspended in 10 mL of acidified methanol. Samples were then stored at –80°C prior analysis.

2.1.3. Extraction of prodelphinidins from redcurrants

Phenolics in freeze-dried redcurrants were extracted three times with methanol and twice with acetone using an Ultra-Turrax homogeniser T25 (same conditions as described above), prior to analysis. The tissue residue in the pellet obtained after centrifugation was acid hydrolysed in order to identify the non-extractable phenolics as described by Maata *et al.* (2001). Six hundred µL of HCl 2M and 1400 µL of methanol were added to the residue and placed in a 3 mL glass V-vial. A teflon coated magnetic stirrer was placed in the vial that was sealed tightly with a PTFE-faced septum prior to heating in a Reacti-Therm heating/stirring module (Pierce, Rockford, IL) for 2 h. Aliquots were then analysed.

2.2. Colorimetric assays

2.2.1. Determination of total phenol content

The total phenol content of the different fruits and vegetables was determined using the Folin-Ciocalteu method of Singleton and Rossi (1965). This method determines phenols and oxidised substances by producing a blue colour from reducing yellow heteropoly phosphomolibdate-tungstate anions. This assay was carried out automatically, using a Gilson 222XL (Gilson Inc, Middleton, Wisconsin, USA) sampling injector and liquid handling system, connected to a Syringe pump 402 and controlled by a 720 Gilson Software, programmed specifically for this effect. In brief 1 mL of 1:10 diluted Folin and Ciocalteu reagent was added to 20 µL of sample and 180 µL of water. After 5 min, 700 µL of a 0.74 M Na_2CO_3 was added; the solutions were mixed and after a 2 h incubation at room temperature, the absorbance was read at 765 nm using a Unicam UV500 UV-visible spectrophotometer (Thermo Spectronic, Cambridge, United Kingdom). The absorbance was calculated in gallic acid equivalents by reference to a standard calibration curve obtained with 50-800 µg/mL gallic acid.

2.2.2. Determination of the antioxidant power using Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay, described by Benzie *et al.* (1996), was used to estimate the antioxidant capacity of fruit and vegetable samples. This method measures the ability of a solution to reduce a ferric-tripyridyl-triazine complex (Fe^{3+}-TPTZ) to the ferrous form Fe^{2+}, producing a blue colour with absorption at 593 nm. The FRAP assay was carried out automatically with the Gilson liquid handling system, as outlined above. Freshly prepared FRAP reagent (containing the Fe^{3+}-TPTZ in excess at pH 3.6), 1.5 mL in volume, was added to 50 µL of sample and 150 µL water. The absorbance at 593 nm was measured 4 min after addition of the reactant. This absorbance was compared to a 0 to 1 mM Fe^{2+} standard curve.

2.2.3. Determination of the antioxidant activity using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) assay adapted to a flow injection system

The $ABTS^{\cdot+}$ assay was based on the method of Pellegrini *et al.* (2003) and was carried in the University of Parma (Department of Public Health). Briefly, the method is based on the capacity of antioxidant molecules to reduce the radical green-blue cation $ABTS^{\cdot+}$ (absorbing at 734 nm) to ABTS which is colourless. Samples were analysed using a flow injection system consisting of a 1100 pump (Hewlett-Packard, Waldbronn, Germany) a Reodhyne injection valve equipped with a 20 µL loop, a single bead string reactor (250 µm x 30 cm x 0.5 mm i.d.) filled with acid-washed silanized beads (Supelco, Bellefonte, PA), a delay coil (0.5 mm i.d., 1.58 mm o.d. and 306 cm in length) and a photodiode array detector (PDA) (Waters Corporation, Milford, MA). The flow of the mobile phase was 0.8 mL/min, and absorbance was recorded at 734 nm. The mobile phase was obtained by dilution in ethanol of a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate. ABTS solution was stored overnight at room temperature in the dark in order to stabilise the radical. Quantification of the antioxidant activity of the solutions injected was achieved by comparing the negative area of the decolourisation peak to the response curve obtained with 10-400 µmol/L of Trolox. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

2.3. HPLC analyses

2.3.1.Determination of ascorbic acid content with an HPLC method.

The ascorbic acid content of fruits and vegetables was assessed as described by Ross (1994). The samples were analysed on a Surveyor HPLC system comprising of a HPLC pump, diode array absorbance detector and an autosampler cooled to 4°C (Thermo Finnegan, San Jose, USA). Separation was carried out using a 5 µm 250 x 4.6 mm i.d. Nucleosil ODS column (Jones Chromatography, Henygoed, Mid Glamorgan, UK) fitted with Supelco guard cartridge C18 and a reversed-phase packing 30-40 µm (Perisorb RP 18, Anachem Ltd, Luton, UK). The column was

eluted isocratically with a mobile phase comprising 0.05 mM sodium hydroxide, 25 mM myristyltrimethylammonium bromide, 0.06 M acetic acid, 7.5 % acetonitrile mobile phase containing 100 mg/L homocysteine and 200 mg/L EDTA. The system was operated at 30°C with a flow-rate of 0.6 mL/min and UV detection at 262 nm. The amount of ascorbic acid was calculated by reference to calibration curve was obtained with 0-500 µM ascorbic acid in 5 % metaphosphoric acid.

2.3.2. Analysis of carotenoids and tocopherols by HPLC

Analysis of carotenoids was carried out using the previously described HPLC equipment. Separation was achieved using a Synergi RP-max column eluted with acetonitrile and ethyl acetate, both containing 2.5 % water, as solvents A and B respectively. Samples were analysed at a flow rate of 1 mL/min with the following solvent programme: 0-2 min: 0% B; 2-12 min: 0-40% B; 12-25 min: 40-60% B.

Identification was carried out by comparison of the retention time of standards. The carotenoids (lutein, α- and β-carotene, lycopene and β-cryptoxanthane) were monitored at 450 nm, while the tocopherols were detected at 290 nm. In both instances the detection wavelength corresponds to the λ_{max}.

2.3.3. On-line measurement of antioxidant activity

2.3.3.1 $ABTS^{\cdot+}$ decolourisation assay for phenolic compounds

The antioxidant activity of fruits and vegetables was measured using an on-line method with the radical cation 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\cdot+}$) as described by Koleva *et al.* (2001) and Dapkevicius *et al.* (2001). The instrumental set-up is schematised in Figure 2.1. Briefly, antioxidants in the the HPLC eluate react post-column with pre-formed $ABTS^{\cdot+}$ and the induced bleaching is measured as a negative peak at 720 nm, which is then converted electronically to a positive peak for ease of integration. The stock solution of $ABTS^{\cdot+}$ was made by adding 0.5 mL of a 70 mM KH_2PO_4 solution to 50 mL of ABTS 2 mM. This mixture was stored overnight in the dark at room temperature to convert ABTS to $ABTS^{\cdot+}$. The $ABTS^{\cdot+}$ stock solution was mixed in a 0.1 M phosphate-buffer solution adjusted to pH 8 in the ratio 1:8 (v/v).

The HPLC system comprised an HPLC pump, an auto-injector with sample cooler, a column oven linked to a photodiode array detector (Thermo Finnegan, San Jose, USA). The separation was carried in the same conditions used for LC-MS analysis (see Section 2.4). $ABTS^{\cdot+}$ solution was delivered by a LC-10-AD *VP* pump, connected to a GT-154 Vacuum Degasser (Shimadzu, Kyoto, Japan) at a flow rate of 0.5 mL/min. After mixing through a 1.5 m x 0.4 mm (i.d.) loop, the absorbance was measured by a UV-detector at 720 nm (Nemphlar Bioscience, Lanark, UK).

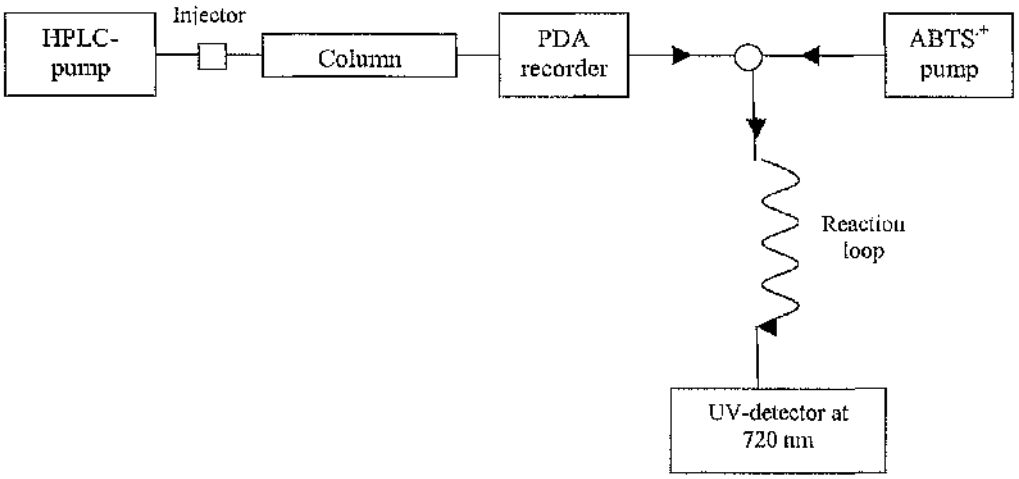


Figure 2.1. Instrument set-up for the HPLC analysis of radical scavenging compounds using an on-line reaction with $ABTS^{\cdot+}$

2.3.3.2. DPPH decolourisation assay for carotenoids and tocopherol

As the mobile phase used to separate carotenoids (mix of ethyl acetate and acetonitrile) was not miscible with the $ABTS^{\cdot+}$ solution, DPPH (1,1-diphenyl-2-picrylhydrazyl) was used instead as a radical solution. The method was adapted from Dapkevicius *et al.* (2001). The general principle was the same but the decolourisation was measured at 515 and 560 nm. A 25 mg/L of DPPH solution was prepared in pure methanol, degassed under vacuum and kept cool on ice during analysis. The DPPH flow rate was 0.5 mL/min, and temperature of the reaction coil was maintained at 40°C.

2.4. Analysis of polyphenolic compounds by HPLC-MS^n

The phenolic profile of fruits and vegetables was analysed on a Surveyor HPLC system comprising an HPLC pump, a PDA detector scanning from 250 to 700 nm and an autosampler cooled to 4°C (Thermo Finnegan, San Jose, USA). Samples were run using a 250 x 4.6 mm (i.d.) Synergie RP-Max column (Phenomenox, Macclesfield, UK) maintained at 40°C. Samples were analysed at a flow rate of 1 mL/min using different solvent gradients (see Table 2.2). After passing through the flow cell of the diode array detector, the column eluate was split and 0.3 mL/min was directed to a LCQ DecaXP ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnegan, San Jose, USA). All samples were analysed in negative ion mode; where samples were found to contain anthocyanin, a second analysis was carried out using positive ion mode.

Table 2.2. Gradient conditions and MS techniques used for the analysis of different fruits and vegetables and biological fluids.

Tissues	Gradient	Ion mode	AH	ISF
Berries	5 to 25 % acetonitrile in 1% formic acid over 60 minutes	+/-	no	no
redcurrants (procyanidin analysis)	5 % isocratic during the first 10 min, and 5 to 30 % acetonitrile in 1% formic acid over 50 minutes	+/-	yes	yes
Fruits and vegetables				
plums, purple broccoli, green pepper	5 to 30 % acetonitrile in 1% formic acid over 60 minutes	+/-	yes	yes
curly kale, broccoli	5 to 30 % acetonitrile in 1% formic acid over 60 minutes	-	yes	yes
clementine, kiwi, cherry tomato, red&yellow pepper	5 to 30 % acetonitrile in 1% formic acid over 60 minutes	-	no	yes
Biological fluid				
plasma, urine	5 to 40 % acetonitrile in 1% formic acid over 60 minutes	+/-	no	no
ileal fluid	5 to 40 % acetonitrile in 1% formic acid over 60 minutes	+/-	yes	no

+: positive ion, -: negative ion, AH: acid hydrolysis, ISF: in source fragmentation

A wide variety of compounds were identified based on their molecular ion and MS^2 fragmentation pattern. In addition, use of a diode array detector also provided absorbance spectra, which give useful information in locating the presence of anthocyanins (λ_{max} 520 nm), flavonols (λ_{max} 365 nm) and phenolic acids, flavanols and procyanidins (λ_{max} 280 nm). The majority of these compounds were found conjugated with various sugars and organic acids. As few standards are available to allow identification by co-chromatography, a variety of different analyses were carried out to confirm their identities. First analysis was by full scan MS^2, which provided information on the molecular ion, type of conjugation and in some case provided information on the aglycone. However, in many instances MS^2 of the aglycone was necessary to aid identification. Analysis of the sample using in source fragmentation (ISF) or after acid hydrolysis provided this information.

2.4.1. Acid hydrolysis of samples

This method hydrolyses conjugated phenolic compounds releasing the aglycone prior to analyses. The method was adapted from Maata *et al.* (2001). 600 µL of HCl 5M were added to and 1400 µL of methanol extracts in a 3 mL glass V-vial. A teflon coated magnetic stirrer was placed in the vial that was sealed tightly with a PTFE-faced septum prior to heating in a Reacti-Therm heating/stirring module (Pierce, Rockford, IL). After 2 h, aliquots were analysed by HPLC-MS^n.

2.4.2. Identification of conjugates by in-source fragmentation

After HPLC, fragmentation was carried inside the source of the mass spectrometer. Collision energy (40%) was applied to the HPLC eluate and any conjugated compounds were broken down to aglycones. This analysis produced the same absorbance trace as an untreated sample but allows for specific identification of the aglycones, without the need for acid hydrolysis.

2.5. Comparison of the antioxidant capacity of plasma after consumption of organic or non organic kiwi juice

2.5.1. Study design

4 healthy volunteers (1 male, 3 female), mean age 35.7 y (range 25 to 46 y), all healthy and non-smokers participated in this study. After an overnight fast, two volunteers were fed with 300 mL of organic kiwi juice, two with conventional juice. Kiwi juices were prepared fresh with 200 g of fruit and 50 g of water, mixed in a blender. During the experiment the volunteers remained fasted. Venous blood samples were taken before the ingestion and after 1, 2, 3 and 4 h. Blood samples were immediately centrifuged at 4000 g at 4°C for 10 min. Plasma was collected and stored at -80°C prior to analysis. One week later the experiment was repeated, the first group was given conventional juice, the second was given the organic juice. Aliquots of kiwi juices were taken each time for FRAP antioxidant measurements.

2.5.2. Measure of the antioxidant activity in plasma samples

Because the reaction was slower in plasma samples, the FRAP method was adapted. The reaction was carried in a water bath at 37°C, and the absorbance at 593 nm was measured in quartz cuvettes 10 min after the addition of the reactant.

2.6. Absorption of redcurrant phenolic compounds in humans

2.6.1. Study design

One subject was recruited through the ileostomy clinic in Glasgow Royal Infirmary. She was female and had undergone ileostomy surgery but was otherwise healthy. Written informed consent was obtained prior the study. The subject followed a flavonoid free diet 2 days before the study. This diet excluded most fruits and vegetables as well as coffee, tea, wine and fruit juice. After an overnight fast 200 g of

red currants with some low fat yoghurt was ingested. Blood samples (6 mL) were collected before the red currant intake and 1, 2, 3, 4, 5 and 24 h after the start of the meal. Blood samples were immediately centrifuged and plasma was stored at -80°C prior to analyses. All ileostomy fluid and urine excreted in the 24 h after the redcurrants consumption were collected, divided into 3 samples: 0-2, 2-5 and 5-24 h. The total volume for urine and quantity for ileal fluid for each period was recorded. The ileal fluid was homogenised and along with urine aliquots were stored at -80°C prior to analysis.

2.6.2. Ileal fluid extraction

Ileostomy fluid homogenate samples (approximately 5 g) were extracted three times with 20 mL of 50% aqueous MeOH in 1% formic acid. For each extraction, samples were homogenised for 1 min with an Ultraturrex, and then shaken at room temperature for 1 h. Samples were then centrifuged at 3000 g for 10 min. Supernatants were combined and reduced to a volume of approximately 5 mL (exact volumes were recorded) *in vacuo*. Samples were stored at -80°C prior to analyses.

2.6.3. Plasma extraction

Plasma samples were treated using the method of Day *et al.* (2001). In order to precipitate plasma proteins, 1.5 mL of acetonitrile was added to 0.5 mL of plasma. Moreover 15 μL of formic acid 50% and 50 μL of ascorbic acid 10 mM was added to stabilised the flavonoids. Samples were vortexed for 30 s every 2 min over a 10 min period. The mixture was then centrifuged for 20 min at 4000 g and supernatant was retained. The precipitate was resuspended in 1.5 mL of methanol and extracted as described for the acetonitrile step. The two supernatants were mixed and dried in a centrifugal vacuum concentrator (Speed vac SPD111v Concentrator, Thermosavant, Holbrook, USA) at 40°C. Samples were resuspended in 50 µL MeOH and 450 µL 1% aqueous formic acid. To calculate the extraction recovery, 10 μL of ^{14}C-quercetin-4-glucoside was used as an internal standard.

2.7. Chemicals

HPLC solvents were obtained from Rathburn Chemicals (Walkerburn, Scotland). (+)-Catechin, quercetin-3-glucoside, myricetin, ellagic acid, kaempferol-3-glucoside, hydroxybenzoic acid, β-carotene, α-tocopherol, trolox, gallic acid, ascorbic acid, metaphosporic acid, DPPH, ferrous sulphate, ferrous chloride, TPTZ, Folin reagent, homocysteine, sodium acetate, myristyltrimethylammonium bromide, sodium hydroxide, ferric ammonium sulphate were obtained from Sigma (Poole, Dorset, UK). ABTS diammonium salt was provided by Merck (Dormstadt, Germany). EDTA and acetic acid were from BDH Chemicals Ltd (Poole, UK), sodium carbonate from Riedel de Haehn GmbH (Seelze, Germany). Naringenin, hesperitin and lutein were purchased from AASC Ltd (Southampton, England); β-cryptoxanthane from Extrasynthese (Genay, France).

2.8. Statistics

Each sample was analysed in triplicate and data are presented as mean values ± standard error (n=3). Where appropriate data were subject to statistical analysis using analysis of variance (ANOVA) to determine the significance of the observed treatment/response relationship. Statistical analyses were performed using Minitab software (Minitab Inc., Addison-Wesley publishing Co, Reading, MA).

CHAPTER 3. COMPARISON OF ANTIOXIDANT ACTIVITY, VITAMIN C AND TOTAL PHENOL CONTENT OF ORGANIC AND CONVENTIONALLY PRODUCED FRUITS AND VEGETABLES AND THEIR *IN VIVO* EFFECT IN HUMANS 41

Chapter 3. Comparison of antioxidant activity, vitamin C and total phenol content of organic and conventionally produced fruits and vegetables and their *in vivo* effect in humans

There is growing consumer interest in organic produce, which anecdotally are thought to be healthier than conventionally produced agricultural crops. In particular, proponents of organic agriculture often claim organically produced plant foods contain higher levels of minerals, vitamins and defence-related secondary metabolites such as phenolic compounds as the use of pesticides and fertilizers is believed to reduce natural disease resistance in conventional products (Brandt and Molgaard, 2001). To date, few studies have investigated the impact of various agricultural practices on the levels of phenolic compounds present in the crops and results are contradictory. In this study, the total phenolic content, as well as the antioxidant activity and the vitamin C content of different fruits and vegetables were measured in both organically or conventionally grown crops. The present study also compared the effect of conventionally and organically produced kiwi fruits on plasma antioxidant activity in humans.

3.1. Comparison of the antioxidant capacity, vitamin C and total phenol content of organic and conventional fruits

Fruits and vegetables analysed were purchased in local supermarkets in Glasgow. The different varieties used in the present study reflect the organic and conventional varieties of fruits and vegetables available on the local market and the composition of the diet as seen from a consumer's perspective.

3.1.1. Measurement of antioxidant capacity of fruits and vegetables

To measure the total antioxidant capacity of organic and conventional tissue extracts, two analytical techniques were used: the FRAP and the TEAC assay. These assays, based on different chemical mechanisms, were selected to take into account the wide variety and range of action of antioxidant compounds present in food materials.

3.1.1.1. Ferric reducing antioxidant power (FRAP)

The antioxidant activity of both organic and conventional fruits and vegetables determined by FRAP is shown in Figure 3.1. All results are expressed in μM/g fresh weight of Fe^{2+} equivalent. Values ranged from 0.4 ± 0.1 in cucumber to 24.0 ± 0.7 in red pepper for organic produce and 0.4 ± 0.1 in cucumber to 21.3 ± 0.6 (in cooked beet root) for conventional crops.

For more than half of the samples tested, namely: apples, kiwi 1, broccoli 2, cauliflower, potato, sweet potato, banana, green bean, cucumber, cooked beet-root and garlic 2, no significant difference in antioxidant activity was observed. Samples of organic carrot, orange, kiwi 2, onion, red pepper, tomato and garlic 1 revealed a trend towards a slightly higher antioxidant activity compared to the conventional produce, whereas for broccoli 1 and pear this trend was reversed. No general trend (measured by ANOVA one-way) could be observed concerning the antioxidant level using the FRAP method between the two different methods of cultivation.

3.1.1.2. 2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) assay adapted to a flow injection system

Antioxidant ability has also been determined using the ability of each extract to reduce the $ABTS^{\cdot+}$ radical. Results are expressed in μmol/g fresh weight trolox equivalents and are shown in Figure 3.2. Profiles obtained are quite different to the results of the FRAP assay. For both organic and non-organic, red pepper showed the highest antioxidant activity, followed by oranges, while bananas presented the lowest.

Similarly to results of the FRAP assay, organic carrot, orange, kiwi 2, onion, red pepper, tomato and garlic 1 revealed a significant trend towards a slightly higher antioxidant activity compared to the conventional produce, whereas for broccoli 1, cooked beet-root and pear this trend was reversed. In this analysis the difference between apple, and pear were as well significantly different ($p<0.05$) with higher content in conventionally grown tissues, whereas for kiwi 1, broccoli 2, cauliflower, potato and green bean it was the contrary, with higher levels in organically grown produce. Again, no general trend (measured by ANOVA one-way) could be observed concerning the antioxidant level between the two different methods of cultivation.

3.1.2. Measurement of ascorbic acid content

The ascorbic acid content was analysed by gradient HPLC with detection at 262 nm as described by Ross (1994). The ascorbic acid content of the organic and conventionally grown fruits and vegetables (expressed in μg/g) are presented in Figure 3.3. No vitamin C was detected in banana, pear and cooked beet-root, independent of the manner of cultivation. Carrot, apple and potato contained very low levels of this vitamin (range 0.2-5.6 μg/g), whereas at the other end of the scale, red pepper contained 511-637 μg/g for conventional and organic culture respectively. If no general trend could be observed in ascorbic acid level (ANOVA one-way), some individual differences were observed among fruits and vegetables. Indeed six organic crops (kiwi 1, cauliflower, potato, red pepper, cherry wine tomato, and garlic 2) contained significantly higher content in vitamin C, whereas for kiwi 2, broccoli 1 and 2, sweet potato and green bean the contrary was true.

3.1.3. Evaluation of phenolic content

Phenolic content was measured using the Folin-Ciocalteu method of Singleton and Rossi (1965). Results were expressed in mg/g of gallic acid equivalents and are presented in Figure 3.4. There was no significant difference in total phenol content of organic or conventionally produced fruits and vegetables, except for three samples, carrot, apple and broccoli 2. Organic carrot and organic broccoli 2 contained significantly higher phenolic content than the conventional varieties, whereas for apple the contrary was true. No general trend could be observed *in vitro* by comparing total phenol content in conventional or organic crops.

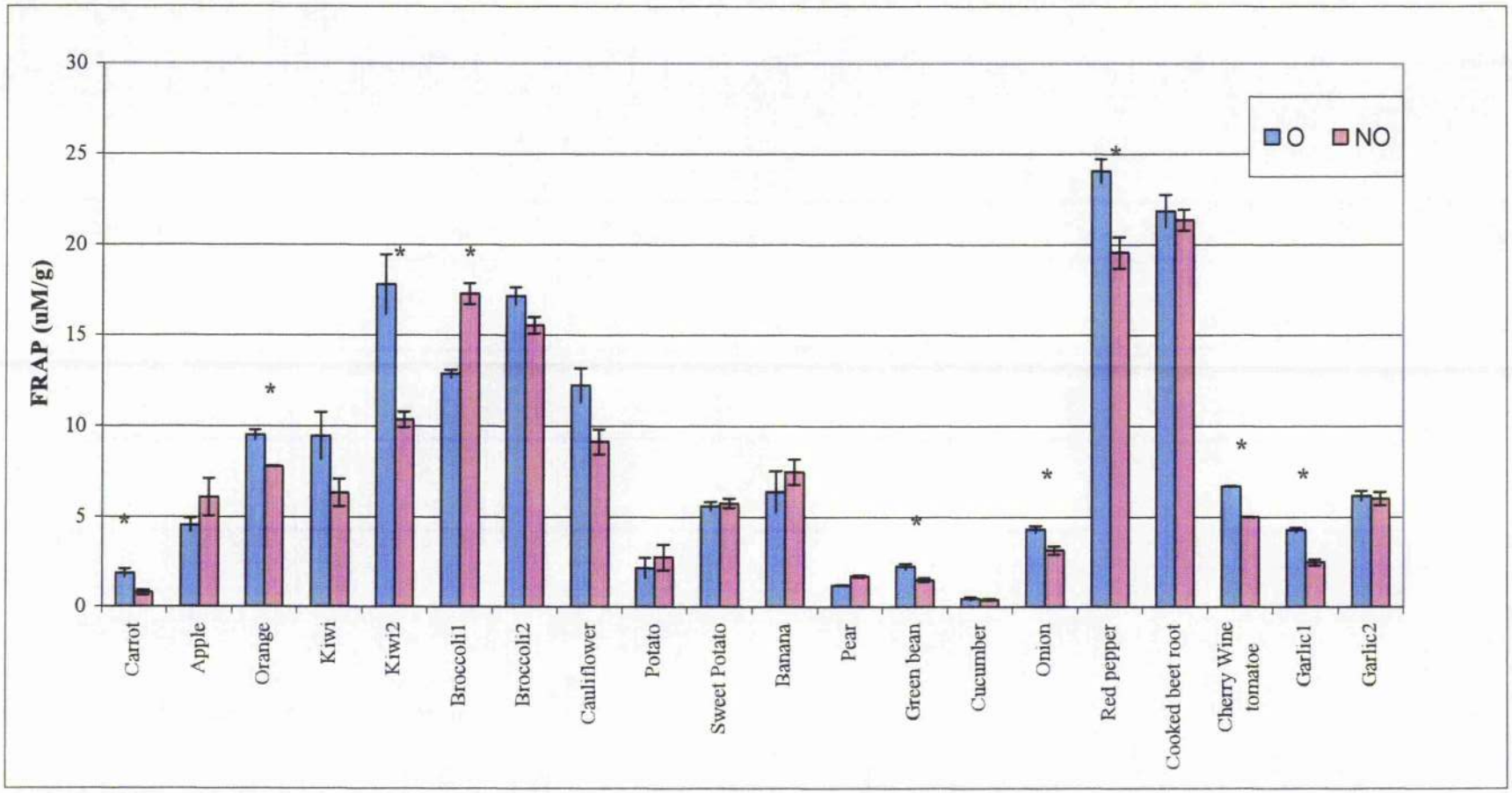


Figure 3.1. Antioxidant activity of organic and non-organic fruits and vegetables by FRAP assay

* differences are significant, $p<0.05$; O, organic produce; NO, conventionally grown produce

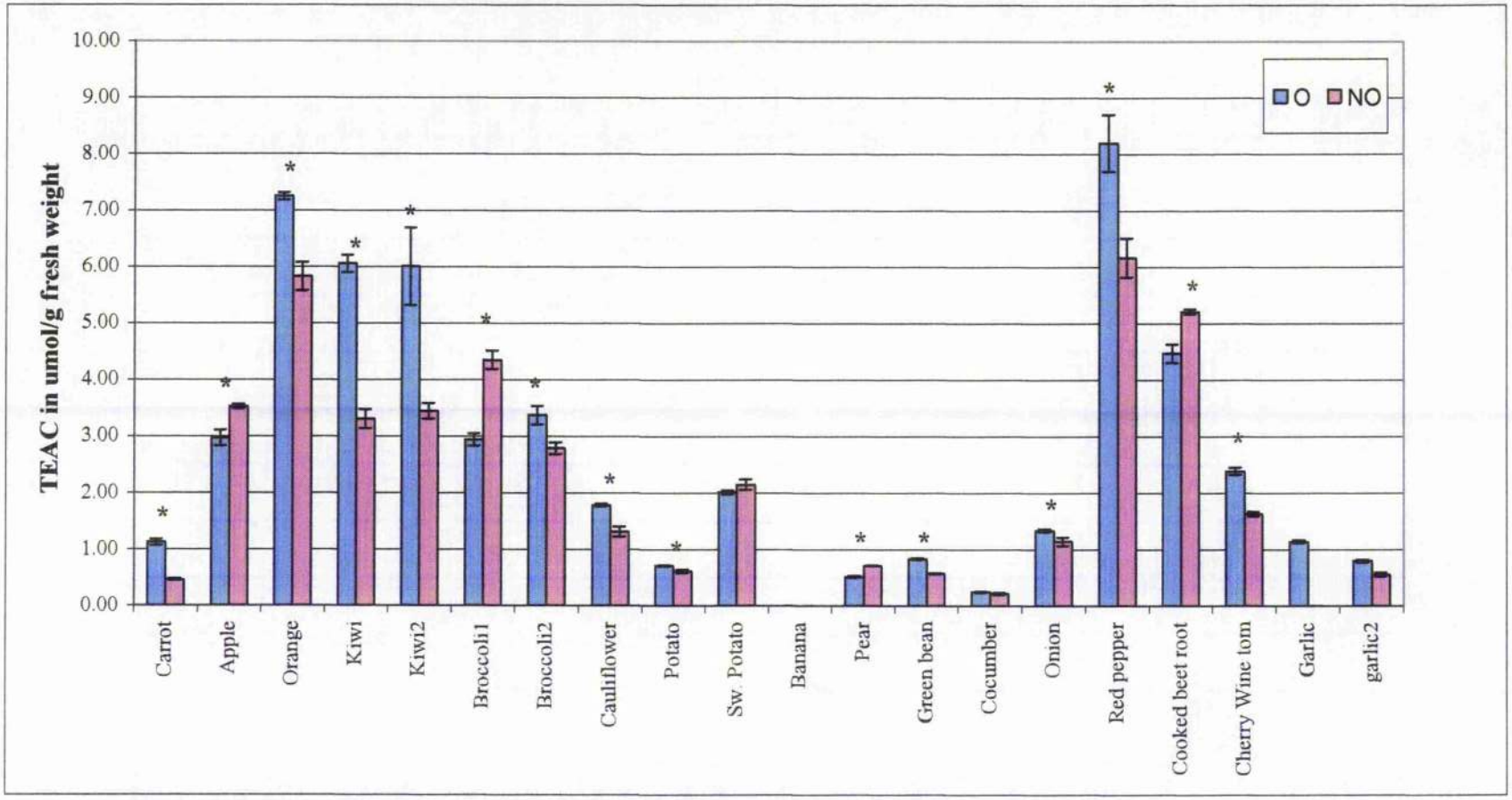


Figure 3.2.. Antioxidant activity of organic and non-organic fruits and vegetables by ABTS assay

* differences are significant, $p<0.05$; O, organic produce; NO, conventionally grown produce

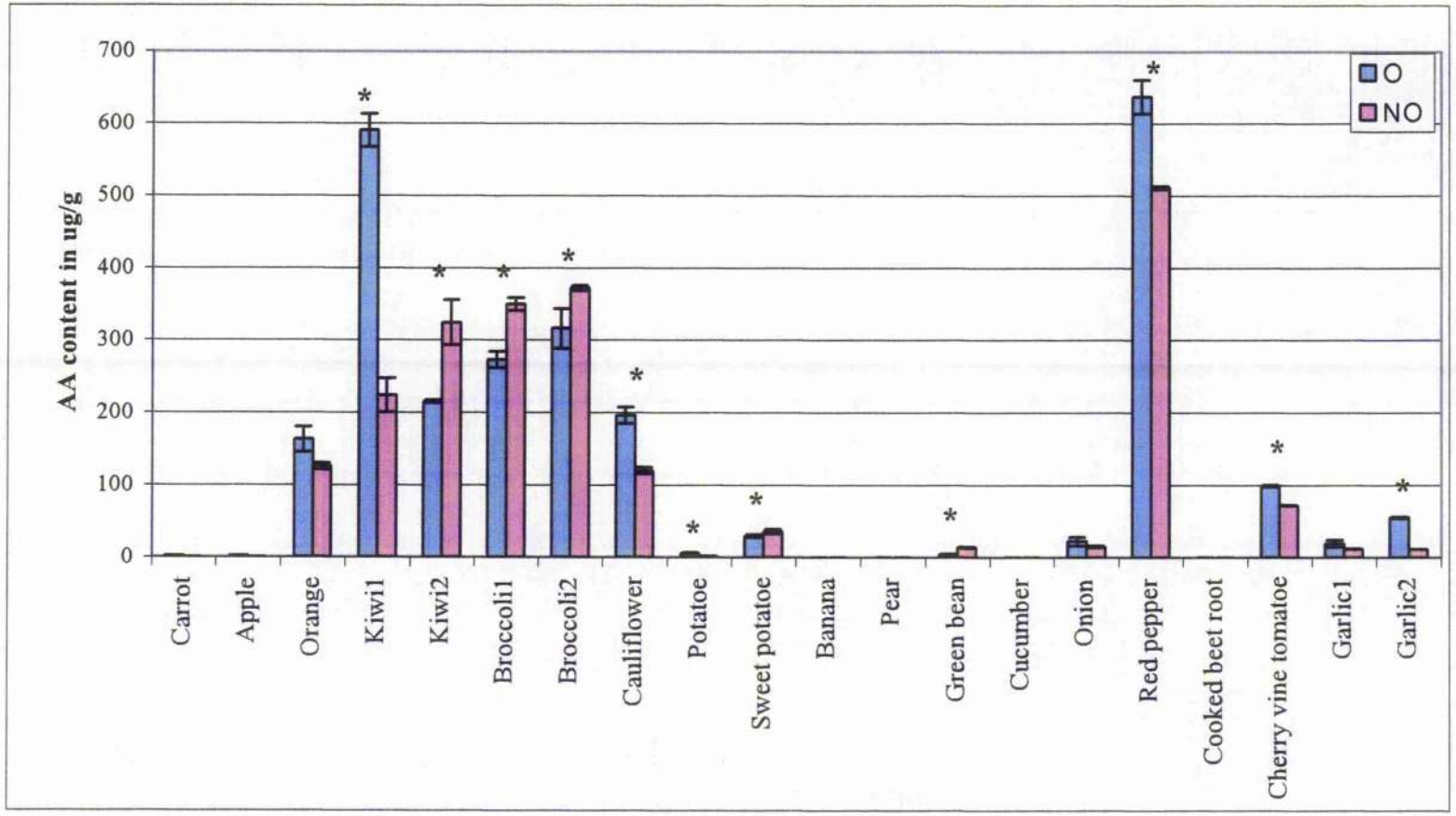


Figure 3.3. Ascorbic acid content of organic and non-organic fruits and vegetables

* differences are significant, $p<0.05$; O, organic produce; NO, conventionally grown produce; AA, ascorbic acid

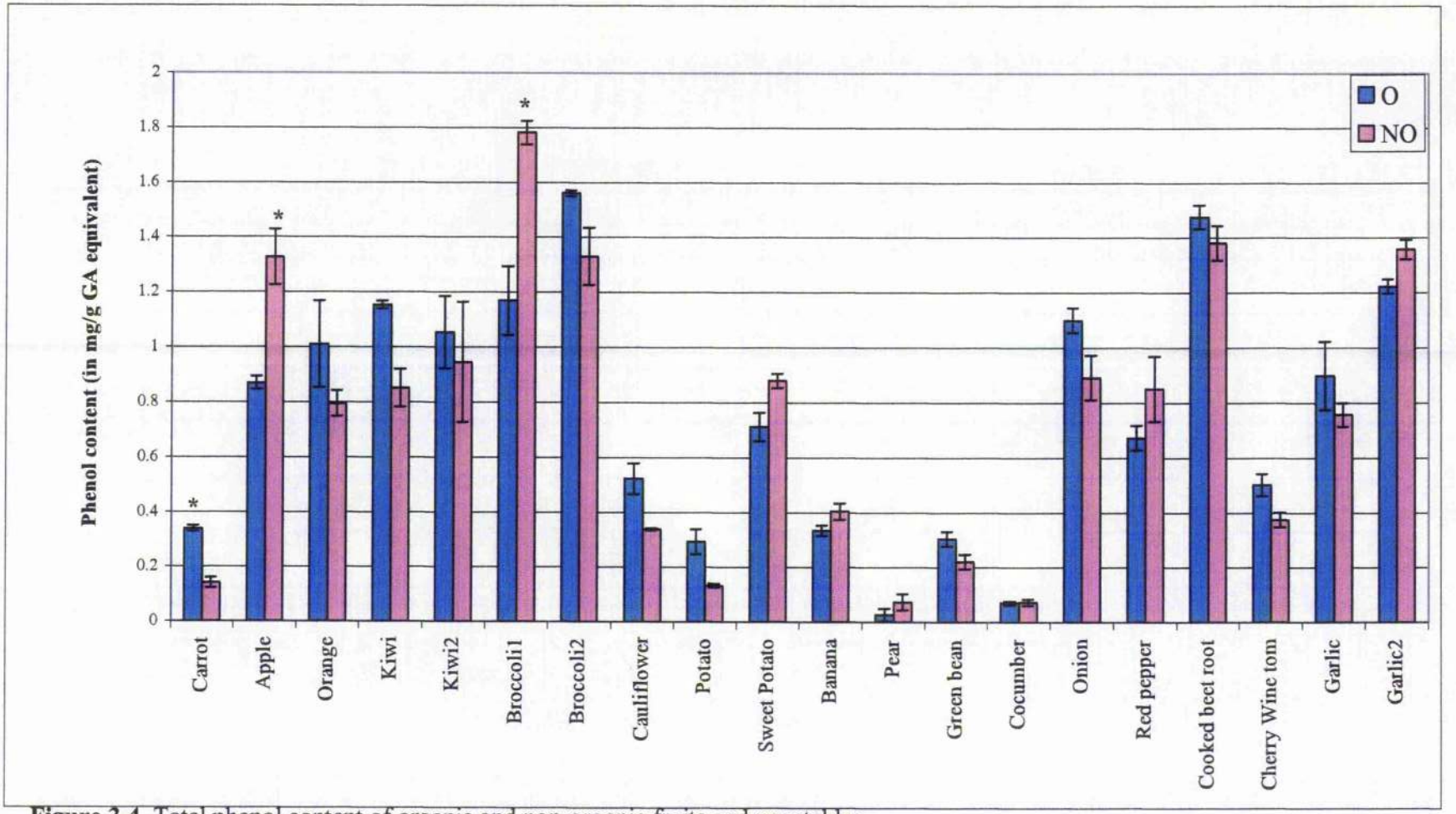


Figure 3.4. Total phenol content of organic and non-organic fruits and vegetables

* differences are significant, $p<0.05$; O, organic produce; NO, conventionally grown produce; GA, gallic acid

3.2. *In vivo* investigations

3.2.1. Feeding study protocol

In order to determine if agricultural practice may have an impact on human health, a crossover feeding study was carried out using kiwi fruits. Four non-smoking healthy volunteers were fed with 300 mL of organic and conventionally grown liquidized kiwi fruits. In each study, organic kiwi fruits exhibited significant higher levels of antioxidant activity compared to conventional varieties (see table 3.1).

Table 3.1. Crossover feeding protocol

		1st week	2nd week
Subject 1 and 3	Nature of kiwi	C_1	O2
	AOX µM/g	(6.57 ± 0.10)	(8.36 ± 0.20)
Subject 2 and 4	Nature of kiwi	O_1	C_2
	AOX µM/g	(7.91 ± 0.09)	(4.88 ± 0.06)

C, conventionnaly grown kiwi; O, organic kiwi; AOX, antioxidant activity, measured by FRAP assay

Venous blood samples were taken at 0, 1, 2, 3 and 4 h after ingestion of the kiwi fruit. After centrifugation plasma was stored at -80°C prior to analysis.

3.2.2. Results

Levels of antioxidants in plasma after the consumption of organic or conventionally produced kiwi fruits was measured using FRAP assay. Results are presented in Figure 3.5. For all subjects and independent of the nature of kiwi fruits consumed, antioxidant activity in plasma peaked 1h to 2h after the consumption of kiwi juice, before to falling to baseline levels by 4h. Data obtained in these feeding studies were subjected to a statistical analysis of variance (ANOVA multifactor). There were significant differences in antioxidant activity between the different subjects and the time of plasma collections, also there was no significant difference between the methods of cultivation of kiwi fruits (organic or conventional).

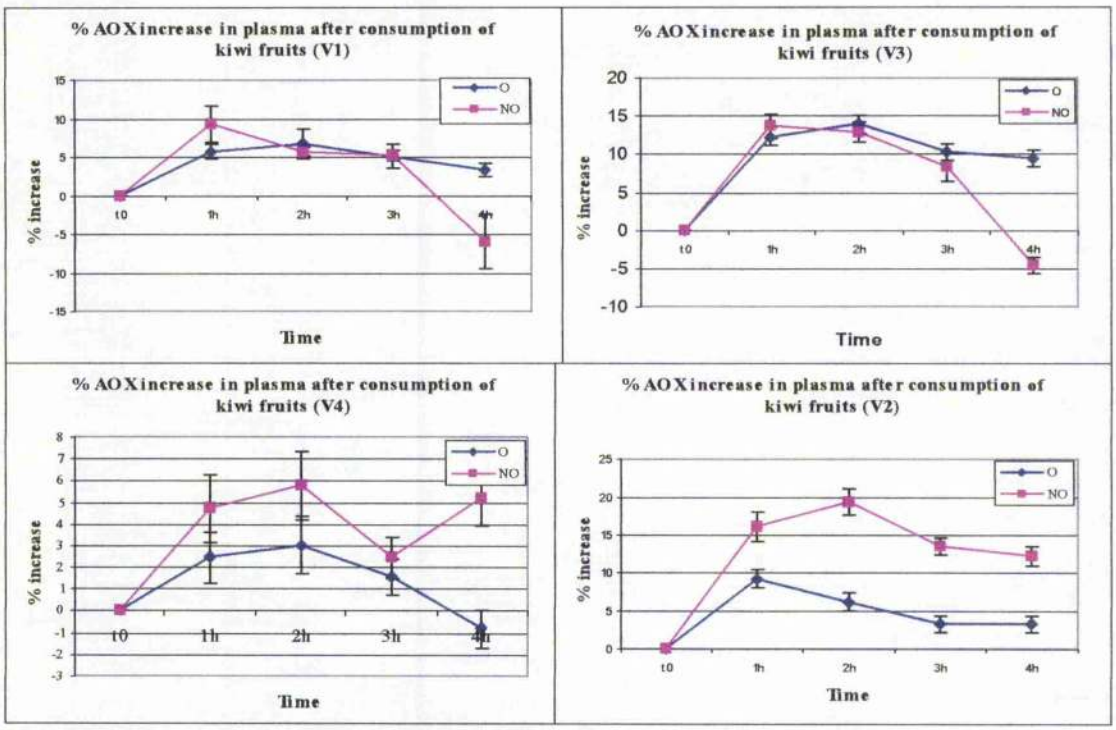


Figure 3.5. Evolution of the antioxidant level in plasma after the consumption of organic or conventionally produced kiwi
O, organic kiwi juice; NO, conventionally grown kiwi juice)

3.3. Discussion and conclusions

Flavonoids and other phenolic compounds are produced by plants in response to viral, microbial infection, insect pressure or stress in the plants (Koes *et al.*, 1994). As no pesticides are used in their production, it was feasible that organic produce could contain more phenolic compounds than the conventional produce. Previously, a number of studies have investigated the levels of phenolics and antioxidants in organic and conventional produce, but results are contradictory and difficult to compare, as study designs vary considerably from study to study (Bourne and Prescott, 2002).

The present study was a "market-orientated supply", tending to monitor the situation of the consumer (Woese *et al.*, 1997). No significant difference was observed between the two groups of produce in total antioxidant capacity, vitamin C and total phenol content. In a recent review (Bourn and Prescott, 2002), results

obtained for studies comparing the nutritional value of organically and conventionally grown food as purchased from retailers were contradictory. Indeed, one reported similar levels of vitamin C in organic and conventional vegetables, whereas another reported vitamin C content to be significantly higher in organic versus conventional produce. Individual differences observed in the present study were inconsistent and reflect probably more the influence of a combination of factors, rather than to the cultivation method alone. Indeed other factors such as stage of maturity, storage conditions, climatic conditions, light exposure and soil type are known to affect the content of bioactive compounds in individual fruits and vegetables (Crozier *et al.*, 1997). Furthermore, in regard of the number of samples analysed, our study is probably not representative of the market as a whole. In order to make any generalized conclusions, there would need to be a large number of studies carried out in different areas with much larger sample group. Nonetheless, the produce analysed in the present study reflect the organic and conventional varieties of fruits and vegetables available on the local market and thus reflect a realistic composition of the diet as seen from a consumer's perspective, independent of other growing factors, which are by the way less easily "controlled" by the consumer.

Previous studies have investigated more specifically the impact of organic cultivation by analysing fruits and vegetables produced in similar conditions. In the review by Bourne and Prescott (2002), analysis of vitamin C content revealed no major differences in four of the studies, while five other studies revealed a trend towards a slightly higher vitamin C content in organically cultivated fruits and vegetables. Concerning the phenolic content, Asami *et al.* (2003) reported a significant trend towards higher levels of total phenolic content in organically produced crops (marionberries, strawberries and corn; same variety, same farm and harvested at the same time to ensure the same degree of ripeness). In other studies, using peaches and apples, there was a trend towards higher levels of antioxidant activity in organically grown produce Carbonaro and Mattera, 2001; Weibel *et al.*, 1998). However, no consistent differences were reported between organic and conventionally cultivated strawberries (Hakkinen *et al.*, 2000).

In vivo, fewer reports are available concerning the impact of organic and conventional diets in humans. Grinder-Penderson *et al.*, (2003) hypothetised that

cultivation conditions may affect the absorption and availability of polyphenolic substances through effects on cell wall structure. They compared conventionally and organically produced diets in a human crossover intervention study. The organic diet contained significantly increased amounts of quercetin and kaempferol compared to the conventional diet, whereas there was a trend toward a higher content of isorhamnetin in the conventional diet. They reported a higher excretion of flavonoids in urine after 22 days of intake of the organically produced diets compared to the conventional one, but no differences in levels of biomarkers of antioxidative status (activity of superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase in erythrocytes and ferric reducing ability of plasma) with the exception of the TEAC measurement, where the values were significantly higher after intake of the conventional diet. In the present study, despite the significantly higher antioxidant content in the organic kiwi fruits compared to the conventional one, the antioxidant activity in plasma in the 5 hours following the consumption of kiwi fruits was not affected by the manner of cultivation of the fruits. This suggests than even when organically grown foods contained higher amounts of antioxidants than foods grown by conventional methods, this increase may not impact on health benefits to humans.

In summary, despite the interest of consumers in organic products, the findings from this study did not show any consistent differences in antioxidant capacity, vitamin C and phenolic content between conventional and organic crops from a consumer point of view. There is even less evidence that organic foods are healthier as compared to foods produced by the traditional methods of cultivation, as far as phenolic compounds and antioxidant levels are concerned. Nonetheless, organically grown foods may contain lower levels of pesticide residues, but again there has been very little documentation of residue levels. To fully evaluate the two food production systems many other aspects such as environmental, economical, social factors may be considered.

CHAPTER 4. ANALYSIS AND IDENTIFICATION OF ANTIOXIDANTS IN BERRIES, FRUITS AND VEGETABLES 54

List of figure

List of table

Chapter 4. Analysis and identification of antioxidants in berries, fruits and vegetables

Diets rich in fruits and vegetables are associated with a reduced risk of cardio-vascular disease and some cancers. Such properties may be due to the presence of antioxidants, and recently attention in this regard has focused on phenolic and polyphenolic compounds. The aim of this study was first to identify and quantify the phenolic compounds as well as vitamin C and carotenoids present in a range of fruits and vegetables, and then to assess the contribution of individual compounds to the total antioxidant activity using HPLC with an on-line detection system.

4.1. Berries

Blueberries, cranberries, blackberries, strawberries, raspberries, blackcurrants, redcurrants were purchased in a local supermarket in Glasgow. No details of varieties were available.

4.1.1 Antioxidant activity, total phenol and vitamin C content

4.1.1.1. Antioxidant activity and total phenol content

The total antioxidant activity (measured by FRAP in μM/g of Fe^{2+} equivalent) as well as the total phenol content (expressed as mg/g of gallic acid equivalents) are presented in Figure 4.1. Dark berries: blackcurrants, blackberries and blueberries have the highest content of phenolic acids (4.1 ± 0.1, 3.6 ± 0.1 and 3.3 ± 0.1 mg/g gallic acid equivalents respectively) as well as higher antioxidant properties (58.3 ± 8.9, 49.8 ± 1.4 and 42.0 ± 3.5 μM/g Fe^{2+} equivalents respectively). Strawberries and redcurrants have lower levels of phenolic compounds and are less powerful in terms of antioxidant activity. Comparison of these values with those reported previously, is difficult as numerous factors influence level of total phenolics and antioxidant activity such as variety, degree of ripeness, season, as well as the solvents used for extraction. Indeed for total phenol content, by using different solvents for extraction Heinonen *et al.* (1998) have reported 3.6-4.3 mg/g for blackberry, 2.7-3.5 mg/g in blueberry, 2.6-3.0 mg/g in raspberry and 1.6-2.9 mg/g in strawberry. For Moyer *et al.* (2002), total phenol values ranged among different species between 2.7-6.8 mg/g in blackberries, 1.3-4.0 mg/g in raspberries and

1.7-9.7 mg/g in blueberries, whereas FRAP values tanging from 40.6-106.1, 13.1-45.2 and 18.5-61.4 µmol/g were obtained for blackberries, raspberries and blueberries respectively.

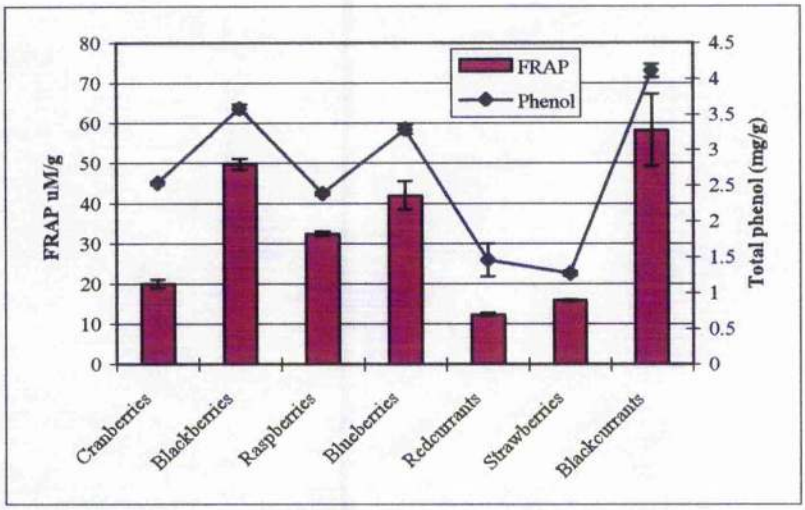


Figure 4.1. Antioxidant activity and total phenol content of berries

4.1.1.2. Vitamin C content

The vitamin C content was analysed by HPLC with detection at 262 nm as described by Ross (1994). The data obtained are presented in Tables 4.18 to 4.24. Blueberries (20.2 µg/g) and blackberries (69.1 µg/g) contain low levels of vitamins as compared to blackcurrants (508 µg/g) or strawberries (397.3 µg/g).

4.1.2. Identification of polyphenols by MS^n analysis

Methanolic extracts of berries were analysed by reversed-phase HPLC using a 60 min, 5-25% acetonitrile in 1% formic acid gradient with the column eluate first directed to a diode array absorbance monitor then to a mass spectrometer with an electrospray interface operating in full scan MS^n mode. Samples were analysed with the mass spectrometer operating in negative and positive ionisation modes.

4.1.2.1. Raspberries

The HPLC traces obtained at 520, 365 and 280 nm facilitated the detection of 16 peaks (Figure 4.3). In addition to the early eluting vitamin C peak, which was quantified in a separate ion-pair HPLC system, MS^n analysis facilitated the identification of 22 phenolic

compounds. Where reference compounds were not available, identifications were made by comparison with published data on raspberry anthocyanins (Barritt and Torre, 1973; Goiffon *et al.*, 1999) and data on the MS fragmentation patterns of anthocyanins and the *m/z* losses associated with cleavage of various sugars and other substituent groups presented by Giusti *et al.* (1999). Similarly, the data of Rommel and Wrolstad (1993) and Zafrilla *et al.* (2001) aided the MS-based identifications of flavonols and ellagic acid sugar conjugates. Previous HPLC-tandem MS analyses of raspberry extracts (Mullen *et al.* 2002a, 2002c, 2003) also assisted the identification of the raspberry phenolics. The identification of phenolic compounds associated with HPLC peaks 1-16 in Figure 4.3, based on MS^2 and MS^3 data, as well as λ_{max}, are summarised below and presented in Table 4.1.

Peak 1 (retention time [t_R] – 17.9 min, λ_{max} – 515 nm) had a positively charged molecular ion ($[M+H]^+$) at *m/z* 611, which on MS^2 produced a fragment ion corresponding to cyanidin at *m/z* 287. The 324 *amu* loss equates with cleavage of a sophorosyl unit, which consists of two glucose moieties. This peak is, therefore identified as cyanidin-3-sophoroside, which is the major anthocyanin in raspberries (Goiffon *et al.*, 1999).

Peak 2 (t_R – 20.0 min, λ_{max} – 515 nm) contained three anthocyanins. One was identified as cyanidin-3-(2^G-glucosylrutinoside) which has been detected in a previous study with raspberries (Mullen *et al.*, 2002a). It had a $[M+H]^+$ at *m/z* 757 which fragmented to produce MS^2 ions at *m/z* 611 ($[M+H]^+$-146, loss of a rhamnosyl moiety) and *m/z* 287 ($[M+H]^+$-470 corresponding to the cleavage of one rhamnosyl and two glycosyl units). The MS of this peak also contained an ion at *m/z* 581 which fragmented on MS^2 to produce a minor ion at *m/z* 449 ($[M+H]^+$-132, loss of a xylosyl group) and a major fragment at *m/z* 287 ($[M+H]^+$-132-162, loss of xylosyl and glucosyl units). This compound is cyanidin-3-sambubioside, a known raspberry anthocyanin (Mullen *et al.*, 2002c). The third anthocyanin was cyanidin-3-glucoside which had a $[M+H]^+$ at *m/z* 449 which on MS^2 yielded a cyanidin fragment ion at *m/z* 287 formed by the cleavage of a 162 *amu* glucosyl unit. Cyanidin-3-glucoside is a known constituent of raspberries (Mullen *et al.*, 2002c).

Peak 3 (t_R – 21.0 min, λ_{max} – 500 nm) was a minor anthocyanin with a $[M+H]^+$ at *m/z* 595 and a MS^2 pelargonidin fragment ion at *m/z* 271 produced by cleavage of a 324 *amu* sophorosyl unit. This compound is therefore identified as pelargonidin-3-

sophoroside detected in raspberries in earlier studies (Goiffon *et al.*, 1999; Mullen *et al.*, 2002c).

Peak 4 (t_R – 22.5 min) contained three components. One had a $[M+H]^+$ at *m/z* 595 which on MS^2 produced a minor fragment at *m/z* 449 ($[M+H]^+$-146, loss of rhamnosyl) and a major fragment at *m/z* 287 (cyanidin, $[M+H]^+$-308, corresponding to cleavage of a rhamnosyl unit and a glucosyl moiety). This compound is, therefore, cyanidin-3-rutinoside. The second compound had a $[M+H]^+$ at *m/z* 727, which yielded MS^2 fragments at *m/z* 581 ($[M+H]^+$-146, loss of a rhamnosyl moiety) and *m/z* 287 (cyanidin, $[M+H]^+$-440, loss of rhamnosyl, glucosyl and xylosyl groups). This corresponds with the fragmentation of cyanidin-3-xylosylrutinoside, a known raspberry anthocyanin (Mullen *et al.*, 2002c). The third component, which had a negatively charged molecular ion ($[M-H]^-$) at *m/z* 289, and produced negatively charged MS^2 fragments at *m/z* 245, 205 and 179, was identified as (-)-epicatechin. This identification was confirmed by co-chromatography with a reference compound.

Peak 5 (t_R – 23.3 min, λ_{max} - 500 nm) also contained three compounds. One had a $[M+H]^+$ at *m/z* 433 which on MS^2 produced a pelargonidin fragment ion at *m/z* 271 via a 162 *amu* cleavage of a glucosyl unit. This compound is pelargondin-3-glucoside, a known endogenous raspberry anthocyanin (Mullen *et al.*, 2002c). The second compound was also an anthocyanin. It had a $[M+H]^+$ at *m/z* 741, which after a 470 *amu* loss corresponding to cleavage of xylosyl and rutinosyl moieties, yielded a pelargonidin MS^2 ion at *m/z* 271. This compound is, therefore pelargonidin-3-2^G-glucosylrutinoside (Mullen *et al.*, 2002c). Negative ionisation of peak 5 revealed the presence of a $[M-H]^-$ at *m/z* 783, which was reported in a previous study to be doubly charged (Mullen *et al.*, 2002a). MS^2 of the doubly charged ion produced a sequence of fragments: *m/z* 1265 (1867-302, loss of a hexahydroxydiphenoyl [HDDP] group), *m/z* 1103 (1867-464, corresponding to the cleavage of an HHDP and a glucosyl unit), *m/z* 933 (1867-934, loss of a HHDP, a glucosyl and a galloyl unit), *m/z* 631 (1867-936, cleavage of an additional HHDP group). On the basis of the MS^2 spectra this peak is probably sanguiin H-10, which was tentatively identified in raspberries in an earlier study (Mullen *et al.*, 2003).

Peak 6 (t_R – 29.1 min, λ_{max} - 250 nm) had a $[M-H]^-$ at *m/z* 1401, reported to be doubly charged, with a true mass of 2804 (Mullen *et al.* 2003). Again MS^2 of the doubly charged ion produced a range of fragments at *m/z* 1869, *m/z* 1567 (1869-302, loss of HHDP), *m/z* 1265, *m/z* 935 and *m/z* 633, which were the same as observed in peak 5 for

sanguiin H-10. This peak, which was one of the main components in the sample, is the ellagitannin lambertianin C, which has previously been detected in extracts of raspberry leaves (Tanaka *et al.*, 1993) and fruit (Mullen *et al.*, 2003).

Peak 7 (t_R – 30.5 min, λ_{max} - 250 nm), the major component in the raspberry extract had a $[M-H]^-$ at *m/z* 1869 which on MS^2 yielded fragment ions very similar to those produced by lambertianin C with ions at *m/z* 1567, 1265, 933 and 631. The mass spectrum and retention properties match those of sanguiin H-6 (Mullen *et al.*, 2002a).

Peak 8 (t_R – 33.8 min, λ_{max} - 360 nm) had a $[M-H]^-$ at *m/z* 433 and MS^2 yielded an ion at *m/z* 301 ($[M-H]^-$-132, loss of a pentosyl unit) and MS^3 produced a *m/z* 257 fragment indicating the presence of ellagic acid rather than quercetin (Mullen *et al.*, 2003). This compound is, therefore, an ellagic acid pentose conjugate.

Peak 9 (t_R] – 35.1min, λ_{max} - 360 nm) produced a $[M-H]^-$ at *m/z* 301 and MS^2 ionisation yielded a fragment at *m/z* 257, which matches the mass spectrum of ellagic acid. This identification was confirmed by co-chromatography with a reference compound.

Peak 10 and 11 (t_R – 37.2 min and 38.2 min, λ_{max} - 340 nm) had identical mass spectra with a $[M-H]^-$ at *m/z* 609, which yielded a MS^2 fragment at *m/z* 301 which in this instance corresponds to loss of hexose and rhamnose units ($[M-H]^-$-308). Peak 10 is, therefore, probably the quercetin galactosylrhamnoside previously detected in raspberries by Mullen *et al.* (2002a). Peak 11 was identified as quercetin-3-glucosylrhamnoside (rutin), and this was confirmed by co-chromatography with a standard. The distinction between these two compounds with identical mass spectra is discussed by Mullen *et al.* (2002a).

Peak 12 (t_R – 38.5min, λ_{max} - 355 nm) is a quercetin hexose, having a $[M-H]^-$ at *m/z* 463, which with neutral loss of 162 *amu* yielded a MS^2 fragment corresponding to quercetin at *m/z* 301. This compound, which eluted before a quercetin-3-glucoside standard, was identified as quercetin-3-galactoside.

Peak 13 (t_R – 39.4 min, λ_{max} - 340 nm) had a $[M-H]^-$ at *m/z* 477 which on MS^2 yielded an ion at *m/z* 301 ($[M-H]^-$-176, loss of a glucuronyl unit) as well as a fragment at *m/z* 179, characteristic of quercetin (Mullen *et al.* 2003). The MS data, and co-chromatography with a standard, thus, indicate the presence of a quercetin-3-glucuronide, which has previously been detected in raspberries by Ryan and Coffin (1971).

Peak 14 (t_R –44.0 min, λ_{max} - 345 nm) had a $[M-H]^-$ at *m/z* 447, with MS^2 producing a major fragment at *m/z* 315 ($[M-H]^-$-132, loss of a pentosyl moiety), as well as a minor fragment at *m/z* 301. Mullen *et al.* (2002a) tentatively identified this component as methyl-ellagic acid pentose conjugate.

Peak 15 and 16 (t_R – 44.1 min and 47.8 min, λ_{max} - 360 nm) had both a $[M-H]^-$ at *m/z* 475, which yielded MS^2 fragments at *m/z* 301 ($[M-H]^-$-174, loss of acetylpentose). Mullen *et al.* (2003) have tentatively identified the earlier eluting peak 15 as ellagic acid-4-acetylxyloside and peak 16 as ellagic acid-4-acetylarabinoside. These compounds previously having been detected in raspberries by Zafrilla *et al.* (2001).

4.1.2.2. Blueberries

Using the same gradient as raspberries, MS^n analysis facilitated the identification of 22 phenolic compounds in blueberries in the 17 peaks of the HPLC trace illustrated in Figure 4.4. The data are summarised in Table 4.2. Previous reports were used to assist the identification of anthocyanins (Goiffon *et al.*, 1991, 1999; Baldo *et al.*, 1995; Prior *et al.*, 2001), in particular for the elution order of anthocyanidins and anthocyanin sugar-conjugates. Indeed, even if the prediction of an absolute order of retention for all anthocyanins is impossible (as the column and the mobile phase affect the retention time), some rules relating retention time to structure have been established by examination of the literature.

i) the elution order of the aglycones is delphinidin, cyanidin, petunidin, pelargonidin, peonidin followed by malvidin (Goiffon *et al.*, 1991).

ii) with the same anthocyanin aglycone moiety, the order of elution is generally 3-galactoside < 3-glucoside < 3-rutinoside < 3-arabinoside(Goiffon *et al.*, 1991). Some exceptions may occur as Van de Casteele *et al.* (1983) reported cyanidin-3-arabinoside eluted between cyanidin-3-galactoside and cyanidin-3-glucoside.

iii) addition of a second sugar moiety to a 3-glucoside anthocyanin generally decreases retention time. But the presence of a methyl group in the rhamnose molecules affects the chromatographic behaviour as cyanidin-3-rutinoside (cyanidin-3-glucosyl-rhamnoside) elutes after cyanidin-3-glucoside (Goiffon *et al.*, 1991).

iv) acylation with acid increases the retention time, the order of elution is 3-glucoside < 3-*O*-acetylglucoside < 3-*O*-(6-*O*-*p*-caffeoyl)glucoside < 3-*O*-(6-*O*-p-coumaroyl)-glucoside (Baldo *et al.*, 1995)

Similarly, the data of Maatta *et al.* (2004) aided the MS-based identifications of flavonols and hydroxycinnamates sugar conjugates. Other studies where polyphenols were identified as aglycones after acid/enzyme hydrolysis (Hakkinen *et al.*, 1999; Justesen *et al.*, 1998, Sellapan *et al.*, 2002) were of limited value in this context.

Peak 1 (t_R – 15.6 min, λ_{max} 520 nm) had $[M+H]^+$ at *m/z* 465, which on MS^2 yielded a fragment ion at *m/z* 303 (delphinidin, $[M+H]^+$-162, loss of a hexosyl unit). On the basis of the mass spectra data and HPLC elution properties, peak 1 was identified as delphinidin-3-galactoside, a major anthocyanin in blueberry (Goiffon *et al.*, 1999; Prior *et al.*, 2001).

Peak 2 (t_R – 19.2 min, λ_{max} – 520 nm) contained two components. One had a $[M+H]^+$ at *m/z* 449 which on MS^2 produced a fragment at *m/z* 287 (cyanidin, $[M+H]^+$-162, corresponding to the cleavage of an hexose unit). This compound, which eluted before a cyanidin-3-glucoside standard, was identified as cyanidin-3-galactoside, a known component of blueberries (Prior *et al.*, 2001; Goiffon *et al.*, 1999). The second compound had a $[M+H]^+$ at *m/z* 435, which yielded on MS^2 fragment at *m/z* 303 (delphinidin, $[M+H]^+$-132, loss of a pentosyl group). This compound was therefore delphinidin-3-arabinoside, also previously identified in blueberries by Goiffon *et al.* (1999) and Prior *et al.* (2001).

Peak 3 (t_R – 18.9 min, λ_{max} – 325 nm) had a $[M-H]^-$ at *m/z* 353 which on MS^2 ionised yielding a major ion at *m/z* 191 and a minor fragment at *m/z* 179. This fragmentation pattern is indicative of the presence of 5-caffeoylquinic acid (Clifford *et al.*, 2003). This hydroxycinnamate, whose identification was confirmed by co-chromatography with a standard, was detected in high concentrations in blueberries in an earlier study by Schuster *et al.* (1985).

Peak 4 (t_R – 21.4 min, λ_{max} – 520 nm) had a $[M+H]^+$ at *m/z* 479 which on MS^2 produced a fragment ion corresponding to petunidin at *m/z* 317 via a 162 *amu* loss of an hexosyl unit. The presence of both petunidin-3-galactoside and petunidin-3-glucoside have been reported in blueberry (Goiffon *et al.*, 1999), but as this compound elutes before cyanidin-3-arabinoside (peak 5), peak 4 is more likely to be petunidin-3-galactoside.

Peak 5 (t_R – 22.5 min, λ_{max} – 515 nm) had a $[M+H]^+$ at *m/z* 419 and a MS^2 cyanidin fragment at *m/z* 287 produced by cleavage of a pentose moiety. This peak is, therefore, identified as cyanidin-3-arabinoside a known blueberry anthocyanin (Goiffon *et al.*, 1999; Prior *et al.*, 2001).

Peak 6 (t_R – 24.7 min, λ_{max} – 520 nm) contained two anthocyanins. One was identified as petunidin-3-arabinoside, which has been identified in blueberry in previous studies (Goiffon *et al.*, 1999; Prior *et al.*, 2001). It had a $[M+H]^+$ at *m/z* 449 which fragmented to produce MS^2 ions at *m/z* 317 (petunidin, $[M+H]^+$-132, loss of a pentosyl unit). The MS of this peak also contained an ion at *m/z* 463 which on MS^2 produced a peonidin fragment at *m/z* 301 ($[M+H]^+$-162, loss of an hexosyl unit). According to elution properties, this compound is probably peonidin-3-galactoside, rather than peonidin-3-glucoside, both of which are blueberry anthocyanins (Goiffon *et al.*, 1999; Prior *et al.*, 2001).

Peak 7 (t_R – 26.7 min, λ_{max} – 520 nm) had a $[M+H]^+$ at *m/z* 493 which on MS^2 produced an ion at *m/z* 331 (malvidin, $[M+H]^+$-162, loss of an hexosyl). On the basis of this mass spectral data, elution prior to malvidin-3-glucoside (see peak 8) and previous studies (Goiffon *et al.*, 1999; Prior *et al.*, 2001) this compound is identified as malvidin-3-galactoside.

Peak 8 (t_R – 27.6 min, λ_{max} – 525 nm) contained two anthocyanins. One, like peak 7, had a $[M+H]^+$ at *m/z* 493 and yielded a similar MS^2 fragmentation pattern. This compound was identified as malvidin-3-glucoside, a known anthocyanin in blueberries (Goiffon *et al.*, 1999; Prior *et al.*, 2001). The identification was confirmed by co-chromatography with a reference compound. The other component had a $[M+H]^+$ at *m/z* 507 which on MS^2 produced a fragment ion corresponding to delphinidin at *m/z* 303. The 204 *amu* loss equates with cleavage of acetyl and glucosyl units. This mass spectral fragmentation pattern is in keeping with the presence of delphinidin-6-acetyl-3-glucoside, an endogenous anthocyanin of lowbush blueberry (Prior *et al.*, 2001).

Peak 9 (t_R – 28.3 min, λ_{max} – 525 nm) was a minor anthocyanin with a $[M+H]^+$ at *m/z* 433 and a MS^2 peonidin fragment at *m/z* 301 produced by the cleavage of a pentosyl unit. On the basis the mass spectrum and previously published data (Goiffon *et al.*, 1999; Prior *et al.*, 2001), this peak is identified as peonidin-3-arabinoside.

Peak 10 (t_R – 30.4 min, λ_{max} – 525 nm) which had a $[M+H]^+$ at *m/z* 463 that fragmented to produce a MS^2 ion at *m/z* 331 ($[M+H]^+$-132, loss of a pentosyl unit) was identified as malvidin-3-arabinoside, which has been detected in blueberries in earlier studies (Prior *et al.*, 2001; Goiffon *et al.*, 1999).

Peak 11 (t_R – 31.8 min, λ_{max} – 355 nm) had a $[M-H]^-$ at *m/z* 479 which with neutral loss of a 162 *amu* hexose unit yielded a MS^2 fragment corresponding to myricetin at *m/z*

317. It was therefore a myricetin-hexose conjugate, probably myricetin-3-galactoside as this compound have been reported previously in blueberries extract (Maatta *et al.*, 2004).

Peak 12 (t_R – 33.5 min, λ_{max} – 525 nm) was a minor anthocyanin. It had a $[M+H]^+$ at *m/z* 521 which fragmented to produce an MS^2 ion at *m/z* 317 ($[M+H]^+$-204, loss of an acetyl and glucosyl unit). This mass spectrum indicated the presence of petunidin-6-acetyl-3-glucoside, a known lowbush blueberry anthocyanin (Prior *et al.*, 2001).

Peak 13 (t_R – 36.8 min, λ_{max} – 325-355 nm) contained two compounds. One had a $[M-H]^-$ at *m/z* 439 which with neutral loss of an 132 *amu* pentose unit yielded a MS^2 fragment corresponding to myricetin at *m/z* 317. It is, therefore, a myricetin-pentose conjugate. The other component had a $[M-H]^-$ at *m/z* 367 which on MS^2 produced fragment at *m/z* 191, 179 and 135. On the basis of MS data, in agreement with the mass spectral key of Clifford *et al.* (2003), this compound was identified as 5-feruloylquinic acid.

Peak 14 (t_R – 38.7 min, λ_{max} – 530 nm) was a minor anthocyanin. It had a $[M+H]^+$ at *m/z* 535 and the same loss of a 204 *amu* as in peak 8 and 12. It was therefore identified as malvidin-6-acetyl-3-glucoside, as its presence detected previously in lowbush blueberry (Prior *et al.*, 2001).

Peak 15 (t_R – 39.1 min, λ_{max} – 355 nm) contained two flavonols. One had a $[M-H]^-$ at *m/z* 609, which on MS^2 yielded a quercetin-like fragment at *m/z* 301. The 308 *amu* loss corresponds to cleavage of a hexose and rhamnose unit. This indicates the presence of quercetin-3-rutinoside, which was confirmed by co-chromatography with a standard. The MS of peak 15 also contained an ion at *m/z* 463, which produced on MS^2 a quercetin fragment at *m/z* 301. This compound, which eluted before a quercetin-3-glucoside standard, was identified as quercetin-3-galactoside, a known constituent of blueberries (Maatta-Riihinen *et al.*, in press)

Peak 16 (t_R – 40.1 - 40.6 min, λ_{max} – 355 nm) contained two flavonols. One was a quercetin hexose conjugate, having a $[M-H]^-$ at *m/z* 463, which with loss of 162 *amu* yielded a MS^2 fragment at *m/z* 301 corresponding to quercetin. It was identified as quercetin-3-glucoside, as it co-eluted with a standard. The second had a $[M-H]^-$ at *m/z* 493, which with loss of 163 *amu* yielded a MS^2 ion at 330. This compound was an unknown flavonol conjugate.

Peak 17 (t_R – 43.9 min, λ_{max} – 355 nm) had a $[M-H]^-$ at *m/z* 433, which yielded a MS^2 fragment at *m/z* 301 ($[M-H]^-$ -132, loss of a pentose unit) and MS^3 at *m/z* 271, 255

and 179. It is, therefore, a quercetin pentose conjugate, possibly quercetin-3-arabinoside, as this compound has previously been identified in blueberries by Maatta-Rihinen *et al.* (in press).

4.1.2.3. Blackcurrants

Using the same gradient, MS^n analysis facilitated the identification of 31 phenolic compounds in 22 HPLC peaks in the blackcurrant trace (Figure 4.5.). The identifications of these peaks, based on a combination of MS^2 spectra, λ_{max}, co-chromatography with reference compounds and previous identification of anthocyanins (Goiffon *et al.*, 1991, 1999; Froytlog *et al.*,1998; Slimestadt *et al.* 2002), flavonols and phenolic acid conjugates (Schuster *et al.*, 1985; Maatta *et al.*, 2003) are discussed below and summarised in Table 4.3.

Peak 1 (t_R – 13.1 min, λ_{max} - 330 nm) had a $[M-H]^-$ at *m/z* 341 and a MS^2 ion at *m/z* 179. The *m/z* 179 fragment is the aglycone caffeic acid produced by a 162 *amu* cleavage of a hexose moiety. On the basis of the mass spectral and UV data and previously published data (Schuster *et al.*, 1985; Maata *et al.*, 2003), this compound is a caffeic acid glucoside.

Peaks 2 and 3 (t_R – 14.7 and 16.1 min, λ_{max} - 520 nm) both had a $[M+H]^+$ at *m/z* 465, which yielded a MS^2 fragment at *m/z* 303 (delphinidin, $[M+H]^+$-162, loss of a hexose moiety). The later eluting peak 3 was identified as delphinidin-3-glucoside and this was confirmed by co-chromatography with a standard. This glucoside is a known component of blackcurrants (Goiffon *et al.*, 1991; Froytlog *et al.*, 1998; Slimestadt *et al.*, 2002). Peak 2 is probably delphinidin-3-galactoside, which would elute before delphinidin-3-glucoside.

Peak 4 (t_R - 18.2 min, λ_{max} - 520 nm) contained three anthocyanins as well as a phenolic acid. One was identified as delphinidin-3-rutinoside, a major anthocyanin in blackcurrants (Goiffon *et al.*, 1999; Slimestad *et al.*, 2002). It had a $[M+H]^+$ at *m/z* 611 which fragmented to produce MS^2 ions at *m/z* 465 ($[M+H]^+$-146, loss of a rhamnosyl moiety) and *m/z* 303 ($[M+H]^+$-308, cleavage of rhamnosyl and glucosyl units). The MS of peak 4 also contained an ion at *m/z* 449, which fragmented on MS^2 to produce an ion at *m/z* 287 ($[M+H]^+$-162, loss of a hexose moiety), characteristic of a cyanidin aglycone. As this compound elutes before cyanidin-3-glucoside (peak 5), it is probably cyanidin-3-

galactoside. The third anthocyanin had a $[M+H]^+$ at *m/z* 435, which on MS^2 produced a fragment at *m/z* 303 (delphinidin, $[M+H]^+$-132, loss of a pentose unit). It co-eluted with "peak 3 in blueberries", previously identified as delphinidin-3-arabinoside. Peak 4 contained 5-caffeoylquinic acid identified on the basis of a $[M-H]^-$ at *m/z* 353 which fragmented yielding a major ion at *m/z* 191 and a minor one at *m/z* 179 in accordance with Clifford *et al.* (2003). This identification was confirmed by co-chromatography with a reference compound.

Peak 5 (t_R – 19.6 min, λ_{max} - 515 nm) had a $[M+H]^+$ at *m/z* 449 which on MS^2 yielded a fragment ion at *m/z* 287 (cyanidin, $[M+H]^+$ -162, loss of a glucosyl unit). On the basis of the mass spectra and co-chromatography with a standard, peak 5 was identified as cyanidin-3-glucoside, a known anthocyanin in blackcurrants (Goiffon *et al.*, 1991; Froytlog *et al.*, 1998).

Peak 6 (t_R – 20.8 min, λ_{max} - 520 nm) was a minor anthocyanin and had a $[M+H]^+$ at *m/z* 479 which yielded a MS^2 fragment ion at *m/z* 317 (petunidin, $[M+H]^+$-162, loss of a hexose unit). According to the retention time (earlier than the petunidin-3-glucoside, see peak 7), this compound is probably petunidin-3-galactoside.

Peak 7 (t_R – 22.1 min, λ_{max} - 515 nm) contain three anthocyanins. One was identified as cyanidin-3-rutinoside as its MS^2 pattern ($[M+H]^+$ at *m/z* 595, which yielded MS^2 fragment ion at *m/z* 449 and 287 produced by the successive losses of rhamnosyl and glucosyl units) matched with this known major blackcurrant anthocyanin (Goiffon *et al.*, 1991; Froytlog *et al.*, 1998). The second anthocyanin had a $[M+H]^+$ at *m/z* 419, which yielded an MS^2 fragment at *m/z* 287 (cyanidin, $[M+H]^+$ -132, loss of a pentose moiety). On the basis of its retention time (similar to peak 5 in blueberries) this compound was tentatively identified as cyanidin-3-arabinoside. The third compound, like peak 6, had a $[M+H]^+$ at *m/z* 479 and yielded a similar MS^2 fragmentation pattern. On the basis of the elution order and previously published data (Slimestadt *et al.*, 2002), this compound is identified as petunidin-3-glucoside.

Peak 8 (t_R – 24.2 min, λ_{max} - 520 nm) contained two anthocyanins. One had a $[M+H]^+$ at *m/z* 463, which on MS^2 yielded a fragment ion at *m/z* 301 (peonidin, $[M+H]^+$-162, loss of an hexose moiety). As this was the earlier eluting of two peonidin hexose conjugates (see peak 9) it was tentatively identified as peonidin-3-galactoside. The second compound had a $[M+H]^+$ at *m/z* 625, which yielded MS^2 fragments at *m/z* 479 ($[M+H]^+$-146, loss of rhamnose) and *m/z* 317 (petunidin, $[M+H]^+$-308, loss of rhamnose

and glucose moieties). This corresponds with the fragmentation pattern of petunidin-3-rutinoside, previously reported to occur in blackcurrants by Slimestadt *et al.* (2002).

Peak 9 (t_R – 25.9 min, λ_{max} - 520 nm) also contained two anthocyanins. One was identified as peonidin-3-glucoside on the basis of its mass spectrum ($[M+H]^+$ at *m/z* 463 and a MS^2 ion at *m/z* 301), its elution order and previous published data (Slimestadt *et al.*, 2002). The MS of this peak also contained an ion at *m/z* 493 which fragmented on MS^2 to produce an ion at *m/z* 331 (malvidin, $[M+H]^+$-162, loss of an hexose unit), tentatively identified as malvidin-3-galactoside because of its elution prior to a further malvidin hexose conjugate, malvidin-3-glucoside (peak 10).

Peak 10 (t_R – 27.7 min, λ_{max} - 525 nm) contained three components. One had a $[M+H]^+$ at *m/z* 493 which with a 162 *amu* cleavage yielded an MS^2 fragment corresponding to malvidin at *m/z* 331. On the basis of co-chromatography with a standard this compound was identified as malvidin-3-glucoside. The second compound had a $[M+H]^+$ at *m/z* 433, which yielded an MS^2 fragment at *m/z* 301 ($[M+H]^+$-132, cleavage of pentosyl unit). It is therefore a peonidin pentose conjugate. The third anthocyanin was identified as peonidin-3-rutinoside, a known component of blackberries (Slimestadt *et al.* 2002), on the basis of a $[M+H]^+$ at *m/z* 609, which yielded a MS^2 peonidin fragment ion at *m/z* 301 formed by the successive cleavage of a rhamnosyl and a glucosyl unit.

Peak 11 (t_R – 29.6 min, λ_{max} - 525 nm) had a $[M+H]^+$ at *m/z* 463, which with a loss of a 132 *amu* pentose unit yielded an MS^2 fragment at *m/z* 331. It is therefore a malvidin pentose conjugate which, as it co-elutes with peak 10 in blueberries, tentatively identified as malvidin-3-arabinoside.

Peak 12 (t_R – 31.6 min, λ_{max} - 355 nm) yielded a $[M-H]^-$ at *m/z* 625 and MS^2 fragments at *m/z* 317 (myricetin, $[M-H]^-$-308, loss of a rutinosyl moiety). It was, therefore, identified as myricetin-3-rutinoside, previously identified in blackcurrant extract by Maatta *et al.* (2003).

Peak 13 (t_R – 32.2 min, λ_{max} - 355 nm) is a myricetin glucuronide conjugate, having a $[M-H]^-$ at *m/z* 493, which with neutral loss of 176 *amu* yielded a MS^2 fragment corresponding to myricetin at *m/z* 317.

Peak 14 (t_R – 35.5 min, λ_{max} - 355 nm) produced a $[M-H]^-$ at *m/z* 521, which fragmented in MS^2 to myricetin at *m/z* 317, through a loss of 204 *amu*. Positive ionisation produced a clearer picture as to the identity of this compound. The molecular ion $[M+H]^+$ was observed at *m/z* 567, which on MS^2 yielded a fragment at *m/z* 319 via a loss of 248

amu. This corresponds to the loss of a malonyglucosyl unit. This loss of 45 from the molecular ion in negative ionisation was also obeserved by Matta *et al.* (2003), who proposed that this came from the loss of the carboxylic function from the malonyl unit. This compound is, therefore, identified as a myricetin-3-malonylglucoside conjugate previously detected in blackcurrants by Maatta *et al.* (2003, 2004).

Peak 15 (t_R – 38.4 min, λ_{max} - 350 nm) had a $[M-H]^-$ at *m/z* 609 which yielded a MS^2 ion at *m/z* 301 (quercetin, $[M-H]^-$-308, loss of a rutinosyl unit). On the basis of this MS spectrum and co-chromatography with a reference compound this compound was identified as quercetin-3-rutinoside which was previously detected in blackcurrants by Maatta *et al.* (2003).

Peak 16 (t_R – 38.7 min, λ_{max} - 350 nm) produced a typical quercetin hexoside mass spectrum ($[M-H]^-$ at *m/z* 463, MS^2 at 301). Peak 15 was tentatively identified as quercetin-3-galactoside because of its elution prior to a further quercetin hexose conjugate, quercetin-3-glucoside (see peak 17).

Peak 17 (t_R – 39.7 min, λ_{max} - 355 nm) contained two compounds. One was a quercetin glucuronide, having a $[M-H]^-$ at *m/z* 477 which with neutral loss of 176 *amu* yielded an MS^2 fragment corresponding to quercetin at *m/z* 301. This compound was identified as quercetin-3-glucuronide on the basis of co-elution with a reference compound. The second component had a $[M-H]^-$ at *m/z* 463, which on loss of 162 *amu* yielded a MS^2 ion at *m/z* 301. This compound was identified as quercetin-3-glucoside; this was confirmed by co-elution with an authentic standard.

Peak 18 (t_R – 42.8 min, λ_{max} - 525 nm) was a minor anthocyanin with a $[M+H]^+$ at *m/z* 611 and a MS^2 delphinidin fragment ion at *m/z* 303 produced by cleavage of a 308 *amu*. This compound is strongly retained on the column. Slimestad *et al.* (2002) have reported the presence of delphinidin conjugate with similar properties (MS^2, absorption and late t_R) identified as delphinidin-3-*O*-(6"-coumaroylglucoside) by co-elution with a standard. This compound is, therefore, identified as delphinidin-3-*O*-(6"-coumaroylglucoside).

Peak 19 (t_R – 43.8 min, λ_{max} - 350 nm) yielding the same $[M-H]^-$ and MS^2 fragments as the myricetin-hexoside-malonate (peak 14), but at *m/z* values 16 *amu* lower indicative of the presence of quercetin-3-malonylglucoside. The occurence of this compound has been reported in blackcurrants by Maatta *et al.* (2003, 2004).

Peak 20 (t_R – 44.6 min, λ_{max} - 315 nm) produced a $[M-H]^-$ at *m/z* 593 and MS^2 fragment at *m/z* 285. The *m/z* 285 ion, produced by a 308 *amu* loss of rhamnose and glucose, corresponded to the aglycone kaempferol. This compound was identified as kaempferol-3-rutinoside, which has been reported to occur in blackcurrants (Maatta *et al.*, 2003). Tjis was confirmed by co-elution with an authentic standard

Peak 21 (t_R – 46.2 min, λ_{max} - 340 nm) was identified as kaempferol-3-glucoside. It yielded a $[M-H]^-$ at *m/z* 447 and an MS^2 fragment at *m/z* 285 after a 162 *amu* loss corresponding to cleavage of a glucose unit. This identification was confirmed by co-chromatography with a standard.

Peak 22 (t_R – 47.9 min, λ_{max} - 360 nm) yielded a $[M-H]^-$ at *m/z* 507 and MS^2 fragments at *m/z* 344 ($[M-H]^-$-162, loss of an hexose unit). Further fragmentation by MS^3, gave ions at *m/z* 316, 301 and 255. This compound was an unknown flavonol conjugate.

4.1.2.4. Cranberries

The HPLC traces obtained at 520, 365 and 280 nm facilitated the detection of 26 phenolic compounds (Figure 4.6). Where standard compounds were not available identifications were based on previously published MS data (Prior *et al.*, 2001) or combinations of MS and NMR (nuclear magnetic resonance) analyses, of cranberry extracts (Vvedenskaa *et al.*, 2004). The identifications of phenolic compounds are summarised in Table 4.4.

Peaks 1, 5 and 14 (t_R – 13.1, 20.2 and 37.7 min, λ_{max} - 280 nm) yielded a $[M-H]^-$ at *m/z* 577 and MS^2 fragments at *m/z* 425 and *m/z* 289 ($[M-H]^-$-288, loss of a catechin/epicatechin unit), characteristic of a procyanidin dimer.

Peaks 2 and 7 (t_R – 15.7 min and 22.2, λ_{max} - 280 nm) both had a $[M-H]^-$ at *m/z* 289 and a MS^2 ion at *m/z* 245. Peak 2 was identified as (+)-catechin and peak 7 as its isomer (-)-epicatechin by co-elution with standards.

Peak 3 (t_R –18.3 min, λ_{max} - 515 nm) contained two components. One had a $[M+H]^+$ at *m/z* 449 which on MS^2 produced a fragment ion corresponding to cyanidin at *m/z* 287. The 162 *amu* loss equates with cleavage of an hexose unit. This compound was, therefore, identified as cyanidin-3-galactoside, one of the major anthocyanins in cranberries (Prior *et al.*, 2001). Negative ionisation of peak 3 revealed the presence of a $[M-H]^-$ at *m/z* 325, which on MS^2 yielded a *m/z* 163 fragment (coumaric acid, $[M-H]^-$-

162, loss of an hexose moiety). This compound is, therefore, a *p*-coumaric acid hexose conjugate, which has been reported previously in cranberries (Maatta *et al.*, 2004)

Peak 4 (t_R –18.7 min, λ_{max} - 325nm) had a $[M-H]^-$ at *m/z* 353 which fragmented yielding a major MS^2 fragment at *m/z* 191 and a minor ion at *m/z* 179, characteristic of 5-caffeoylquinic acid (Clifford *et al.*, 2003). This identification was confirmed by co-chromatography with a standard.

Peak 6 (t_R –21.9 min) contained two components. One had a $[M+H]^+$ at *m/z* 419 which on MS^2 produced a fragment ion corresponding to cyanidin at *m/z* 287 via a 132 *amu* cleavage of a pentose unit. This compound was identified as cyanidin-3-arabinoside, a major anthocyanin present in cranberries (Prior *et al.*, 2001). The other component had a $[M-H]^-$ at *m/z* 1725 which on MS^2 yielded a series of fragments, including *m/z* 1437, *m/z* 1149, *m/z* 861 and *m/z* 573, corresponding to the sequential loss 1, 2, 3 and 4 catechin/epicatechin units. This compound was identified as a procyanidin hexamer. Prior *et al.* (2001) reported the presence of procyanidin monomers to heptamers, doubly or singly linked, in cranberries.

Peak 8 (t_R –24.2 min, λ_{max} - 515 nm) was a major anthocyanin with a $[M+H]^+$ at *m/z* 463 and a MS^2 peonidin fragment at *m/z* 301 produced by cleavage of a hexose moiety. This fragmentation pattern is in keeping with the presence of peonidin-3-galactoside, a known constituent of cranberries (Prior *et al.*, 2001).

Peak 9 (t_R –26.2 min, λ_{max} - 280 and 520 nm) contained two components. One had a $[M+H]^+$ at *m/z* 593, which on MS^2 produced a fragment ion corresponding to malvidin at *m/z* 331. This anthocyanin was identified as malvidin-3-glucoside as it co-eluted with a reference compound. With negative ionisation this peak also contained a $[M-H]^-$ at *m/z* 867 which fragmented on MS^2 yielding ions at *m/z* 1201, 577 and 289 (catechin unit). The presence of an ion at *m/z* 1201 suggests this compound is multicharged and it appear to be a polymeric procyanidin.

Peak 10 (t_R –27.9 min, λ_{max} - 515 nm) had a $[M+H]^+$ at *m/z* 433, which yielded MS^2 fragments at *m/z* 301(peonidin, a $[M+H]^+$-132, loss of a pentosyl unit). It was therefore identified as a peonidin-pentoside.

Peak 11 (t_R –29.9 min, λ_{max} - 520 nm) was a minor anthocyanin and had a $[M+H]^+$ at *m/z* 463 which with neutral loss of 162 *amu* yielded a MS^2 fragment corresponding to malvidin at *m/z* 331. This compound was a malvidin-pentose conjugate,

possibly malvidin-3-arabinoside based on its co-elution with peak 10 in blueberries (see Figure 4.4, Table 4.2).

Peak 12 (t_R –31.3 min, λ_{max} - 355 nm) contained two phenolics. One produced a $[M-H]^-$ at *m/z* 479 which with a loss of 162 *amu* yielded a MS^2 at *m/z* 317. It was therefore a myricetin hexose conjugate, probably myricetin-3-galactoside a known cranberry flavonol (Vvedenskaya *et al.*, 2004). The other component was identified as an A-type procyanidin dimer on the basis of its mass spectrum. It had a $[M-H]^-$ at *m/z* 575 which produced MS^2 fragments at *m/z* 449 and 289 (catechin monomer).

Peak 13 (t_R –36.2 min, λ_{max} - 355 nm) contained two components. One was again multicharged, with a $[M-H]^-$ at *m/z* 863 producing ions at *m/z* 1151 and 575 in keeping with the fragmentation of a procyanidin polymer. The second compound was a myricetin pentose conjugate having a $[M-H]^-$ at *m/z* 449 and a MS^2 ion at *m/z* 317. This compound may be myricetin-3-arabinoside, which has been identified in cranberries by Vvedenskaya *et al.* (2004).

Peak 15 (t_R –38.6 min, λ_{max} - 355 nm) also contained two compounds. One was the major flavonol present in cranberries and produced a mass spectrum characteristic of a quercetin hexose conjugate ($[M-H]^-$ at *m/z* 463, MS^2 ion at *m/z* 301 and MS^3 of this ion produced a major fragment at *m/z* 179). In view of this mass spectrum, the high concentration and HPLC order of elution, this compound is probably quercetin-3-galactoside previously detected in cranberries by (Vvedenskaya *et al.*, 2004). Peak 15 also contains a procyanidin trimer based on a $[M-H]^-$ at *m/z* 865 which yielded MS^2 fragments at *m/z* 577 and 289.

Peaks 16, 17 and 18 (t_R – 42.2, 43.4 and 44.0 min, λ_{max} - 355 and 350 nm) all had a $[M-H]^-$ at *m/z* 433 which with neutral loss of a 132 *amu* pentose unit yielded a MS^2 fragment corresponding to quercetin at *m/z* 301. These three flavonols are, therefore, all quercetin pentose conjugates. Based on their elution order they may be respectively quercetin-3-xylopyranoside, quercetin-3-arabinopyranoside and quercetin-3-arabinofuranoside previously identified in a cranberry extract by HPLC-MS and NMR (Vvedenskaya *et al.*, 2004).

Peak 19 (t_R - 46.5 min, λ_{max} - 345 nm) had a $[M-H]^-$ at *m/z* 447 which yielded an MS^2 fragment at *m/z* 301 (quercetin), the $[M-H]^-$-146 loss corresponding to cleavage of a rhamnose unit. The mass spectral data and HPLC retention are in keeping with peak 19

being quercetin-3-rhamnoside, which was previously detected in cranberries by Vvedenskaya *et al.* (2004).

4.1.2.5. Blackberries

HPLC-MS^n analysis facilitated the identification of 15 phenolic compounds in blackberries (see figure 4.7 and Table 4.5). Where standards were not available, identifications were assisted by data presented in published information on blackberry anthocyanins (Dugo *et al.*, 2001; Goiffon *et al.*, 1991) and HPLC-tandem MS analyses of raspberry ellagitannins (Mullen *et al.* 2002a, 2003).

Peak 1 (t_R – 19.5 min, λ_{max} - 505 nm) was the main phenolic present in blackberries and was identified as cyanidin-3-glucoside. It had a $[M+H]^+$ at *m/z* 449 which with loss of 162 *amu* yielded a cyanidin MS^2 ion at *m/z* 287. The identification was confirmed by co-elution with an authentic standard.

Peak 2 (t_R –22.0 min, λ_{max} - 515 nm) produced a $[M+H]^+$ at *m/z* 595 and MS^2 yielded ions at *m/z* 449 ($[M+H]^+$-146, loss of a rhamnosyl group) and 287($[M+H]^+$ -308, loss of rhamnose and glucose units). Peak 2 was identified as cyanidin-3-rutinoside, a known anthocyanin in blackberry (Goiffon *et al.*, 1991; Dugo *et al.*, 2001).

Peak 3 (t_R –23.5 min, λ_{max} - 250 nm) revealed the presence of a $[M-H]^-$ at *m/z* 783 which was doubly charged as on MS^2 it fragmented yielding ions at *m/z* 1265, 1104, 933 and 633. This compound could be sanguiin H-10, as it had the same MS^2 fragmentation and retention time as the raspberry ellagitanin sanguiin H-10 (see Table 4.1, peak 5).

Peak 4 (t_R – 27.3 min, λ_{max} - 250 nm) had a $[M-H]^-$ at *m/z* 1869 which on MS^2 produced a sequence of fragments: *m/z* 1567 ($[M-H]^-$-303, loss of an [HHDP] group), *m/z* 1265 ($[M-H]^-$-605, loss of a second [HHDP] group), *m/z* 933 ($[M-H]^-$-937, further cleavage of glucosyl and galloyl moieties) and *m/z* 631 ($[M-H]^-$-1239, loss of an additional [HHDP] group). This corresponds to the MS^2 fragmentation pattern of dimeric ellagitannins.

Peak 5 (t_R – 28.8 min, λ_{max} - 250 nm) was tentatively identified as lambertianin C, as its properties matched those of the ellagitannin detected in raspberries (Figure 4.3, Table 4.1, peak 6) and previously identified by Mullen *et al.* (2003).

Peak 6 (t_R – 29.6 min, λ_{max} - 535 nm) had a $[M+H]^+$ at *m/z* 535 which on MS^2 produced a fragment ion corresponding to cyanidin at *m/z* 287. The 248 *amu* loss

corresponds to the cleavage of a malonylglucose unit. This peak is, thus, tentatively identified as a cyanidin malonylglucoside.

Peak 7 (t_R –30.2 min, λ_{max} - 250 nm) was tentatively identified as sanguiin H-6, as the mass spectrum and retention properties match those of sanguiin H-6 in raspberries (Figure 4.3, Table 4.1, peak 7) (Mullen *et al.* 2003).

Peak 8 (t_R – 31.8 min, λ_{max} - 515 nm) was a minor anthocyanin with a $[M+H]^+$ at *m/z* 593, which fragmented on MS^2 to produce ions at *m/z* 449 ($[M+H]^+$-146, loss of a rhamnose unit) and *m/z* at 287 (cyanidin, $[M+H]^+$-308, loss of rhamnose and glucose moieties). It was therefore identified as a cyanidin-rutinoside.

Peak 9 (t_R – 35.3 min, λ_{max} 365 nm) had a $[M-H]^-$ at *m/z* 769 and MS^2 yielded ions at *m/z* 463 ($[M-H]^-$-306), 505 ($[M-H]^-$-264) and 301 ($[M-H]^-$-468). MS^3 of the *m/z* 301 ion produced a *m/z* 257 fragment indicating the presence of ellagic acid rather than quercetin. This compound is therefore an unknown conjugate of ellagic acid.

Peak 10 (t_R – 36.8 min, λ_{max} - 355 nm) had a $[M-H]^-$ at *m/z* 609, which with neutral loss of 308 *amu* yielded MS^2 fragments at *m/z* 301 and *m/z* 179. This indicates the presence of a quercetin rutinoside but not quercetin-3-rutinoside, which had a later HPLC retention time.

Peak 11 (t_R – 38.2 min, λ_{max} - 350) had a $[M-H]^-$ at *m/z* 463, which yielded a MS^2 fragment at *m/z* 301 corresponding to the loss of a hexose unit. Peak 11 was identified as a quercetin-3-galactoside, as it eluted prior to another quercetin hexose conjugate, qurectin-3-glucoside (see peak 12).

Peak 12 (t_R –3 9.1 min, λ_{max} 355 nm) contained two compounds. One is a quercetin glucuronide, having a $[M-H]^-$ at *m/z* 477, which with the loss of a 176 *amu* glucuronide unit yielded an MS^2 fragment at *m/z* 301. Co-chromatography with a reference compound established that it was quercetin-3-glucuronide. The other component had $[M-H]^-$ at *m/z* 463, which produced a MS^2 quercetin fragment at *m/z* 301. On the basis of co-elution with a standard, this compound was identified as quercetin-3-glucoside.

Peak 13 (t_R – 43.2 min, λ_{max} - 345 nm) produced a $[M-H]^-$ at *m/z* 505 which with loss of 204 *amu* yielded an MS^2 fragment corresponding to quercetin at *m/z* 301. As in peak 14 in blackcurrants (see table 4.3), the 204 amu loss corresponds to the loss of hexose and malonyl groups, which in negative ion mode lost the 45 *amu* carboxylic function from the pseudomolecular ion (Maatta *et al.*, 2003). This compound was tentatively identified as a quercetin hexose malonate conjugate.

Peak 14 (t_R – 45.8 min, λ_{max} - 345 nm) had a $[M-H]^-$ at *m/z* 447, with MS^2 producing a fragment at *m/z* 285 (kaempferol, $[M-H]^-$-176, loss of a glucuronyl moiety). It was, therefore, a kaempferol glucuronide conjugate.

4.1.2.6. Strawberries

The HPLC traces obtained at 520, 365 and 280 nm facilitated the detection of 11 phenolic compounds (Figure 4.8). Anthocyanin identifications were assisted by previously published data (Goiffon *et al.*, 1991 and 1999; Tamura *et al.*, 1995; Bridle and Garcia-Viguera, 1997) and the identification of hydroxybenzoic acid and hydroxycinnamate conjugates was aided by the report of Schuster *et al.*, 1985. The data are summarised in Table 4.6.

Peaks 1 and 2 (t_R – 13.3 min and 14.3 min, λ_{max} - 280 nm) yielded a $[M-H]^-$ at *m/z* 577 and MS^2 fragments at *m/z* 289 ($[M-H]^-$-288, loss of catechin/epicatechin), characteristic of B-type procyanidin dimers.

Peak 3 (t_R – 15.7 min, λ_{max} - 280 nm) was identified as a procyanidin trimer, with a $[M-H]^-$ at *m/z* 865, producing MS^2 fragments at *m/z* 577 and 289, with each 288 amu loss representing cleavage of a catechin/epicatechin monomer.

Peak 4 (t_R – 18.4 min, λ_{max} - 325 nm) revealed the presence of a $[M-H]^-$ at *m/z* 325 and MS^2 at *m/z* 163 ($[M-H]^-$-162, loss of an hexose moiety). This is in keeping with the presence of a *p*-coumaric acid hexose conjugate which has been previously detected in strawberries (Schuster *et al.*, 1985).

Peak 5 (t_R – 23.5 min, λ_{max} - 500 nm) is the major anthocyanin present in strawberries. It had a $[M+H]^+$ at *m/z* 433 which with the loss of 162 *amu* yielded an MS^2 fragment corresponding to pelargonidin at *m/z* 271. This peak was identified as pelargonidin-3-glucoside, a known major anthocyanin in strawberry (Goiffon *et al.*, 1991; Bridle and Garcia-Viguera, 1997).

Peak 6 (t_R – 33.6 min, λ_{max} - 500 nm) produced a $[M+H]^+$ at *m/z* 519, which on MS^2 yielded a pelargonidin fragment at *m/z* 271 via a 248 *amu* cleavage of a malonylglucosyl unit. It was therefore identified as a pelargonidin malonylglucoside conjugate, possibly pelargonidin-3-*O*-(6-*O*-malonylglucoside) which has been identified by Tamura *et al.* (1995).

Peak 7 (t_R – 34.9 min, λ_{max} - 375 nm) had a $[M-H]^-$ at *m/z* 447 and MS^2 yielded an ion at *m/z* 301 ($[M-H]^-$-146, loss of a rhamnosyl unit). MS^3 of the *m/z* 301 ion produced a *m/z* 257 fragment indicating the presence of an ellagic acid rather than a quercetin conjugate. This compound was, therefore, an ellagic acid rhamnoside.

Peak 8 (t_R – 37.9 min, λ_{max} - 250 nm) had a $[M-H]^-$ at *m/z* 1870 which on MS^2 produced fragments at *m/z* 1567 ($[M-H]^-$-303, loss of an HHDP group), *m/z* 1265 ($[M-H]^-$ -605, loss of an additional HHDP group), *m/z* 935 ($[M-H]^-$-937, further cleavage of glucosyl and galloyl moieties) and *m/z* 631 ($[M-H]^-$-1239, additional loss of an HHDP group). This fragmentation pattern indicates the presence of a dimeric ellagitannin (Mullen *et al.* 2003), possibly a sanguiin type.

Peak 9 (t_R – 39.5 min, λ_{max} - 355 nm) had a $[M-H]^-$ at *m/z* 477 which on MS^2 yielded an ion at *m/z* 301 ($[M-H]^-$ -176, loss of a glucuronyl unit) as well as a fragment at *m/z* 179, characteristic of quercetin. This mass spectrum together with co-chromatography with a reference compound established the identity of quercetin-3-glucuronide.

Peak 10 (t_R – 46.0 min, λ_{max} - 340 nm) had a $[M-H]^-$ at *m/z* 447 which with the loss of 162 *amu* yielded a MS^2 ion at *m/z* 285. This mass spectrum and co-elution with a standard identified peak 10 as kaempferol-3-glucoside.

Peak 11 (t_R – 50.9 min, λ_{max} - 345 nm) yielded a $[M-H]^-$ at *m/z* 489, which with neutral loss of 204 *amu* yielded an MS^2 fragment corresponding to kaempferol at *m/z* 285. The 204 *amu* loss corresponds to cleavage of hexose and malonyl groups, which in negative ion mode lost the carboxylic function (mass unit 45) from the pseudomolecular ion (Maatta *et al.*, 2003), as seen in the blackcurrant extract. This compound was tentatively identified as a kaempferol malonylhexose conjugate. Withopf *et al.* (1997) reported that malonylation of glycoconjugates is a common pathway in plant secondary metabolism in strawberries.

4.1.2.7. Redcurrants

The HPLC traces obtained at 520, 365 and 280 nm revealed the presence of 12 peaks in which 13 compounds were detected by tandem MS (Figure 4.9). Where standard compounds were not available identifications were assisted by previous analyses of anthocyanins (Oydvin, 1974; Goiffon *et al.*, 1991 and 1999; Maatta *et al.*, 2001 and

2003) and hydroxycinnmate conjugates in redcurrants (Schuster *et al.*, 1985). The identification of phenolic compounds are summarised below and presented in Table 4.7.

Peak 1 (t_R – 9.2 min, λ_{max} - 260 nm) had a $[M-H]^-$ at *m/z* 299 and MS^2 yielded an ion at *m/z* 137 ($[M-H]^-$-162, loss of an hexose unit). This compound was tentatively identified as a 4-hydroxybenzoic acid hexose conjugate as, in agreement with Matta *et al.* (2001), acid hydrolysis released 4-hydroxybenzoic acid.

Peaks 2 and 3 (t_R – 11.2 min and 16.1, λ_{max} - 330 nm) had both a $[M-H]^-$ at *m/z* 341 which on MS^2 yielded a fragment releasing an ion at *m/z* 179. The *m/z* 179 ion is the aglycone caffeic acid produced by a loss of 162 *amu*, which corresponds with the cleavage of a hexose moiety. On the basis of the mass spectral and UV data and previously published data (Schuster *et al.* 1985), both of these compounds were identified as caffeic acid hexose conjugates.

Peak 4 (t_R – 20.3 min, λ_{max} - 515 nm) had a $[M+H]^+$ at *m/z* 581 which on MS^2 produced a cyanidin fragment ion at *m/z* 287. The loss of 294 *amu* corresponds to the cleavage of xylosyl and glucosyl units. The mass spectrum and the HPLC retention correspond to that of cyanidin-3-sambubioside, identified in redcurrants in earlier studies (Oydvin, 1974; Maatta *et al.*, 2003).

Peak 5 (t_R – 22.0 min, λ_{max} - 515 nm) was the main peak present in the redcurrant extract and contained two compounds. The first had a $[M+H]^+$ at *m/z* 595 which on MS^2 produced a minor fragment at *m/z* 449 ($[M+H]^+$-146, loss of rhamnosyl unit) and a major fragment at *m/z* 287 (cyanidin, $[M+H]^+$-308 corresponding to the cleavage of rhamnosyl and glucosyl moieties). This compound was, therefore, cyanidin-3-rutinoside. The second compound had a $[M+H]^+$ at *m/z* 727, which yielded MS^2 fragments at *m/z* 581 ($[M+H]^+$-146, loss of rhamnose) and *m/z* 287 (cyanidin, $[M+H]^+$-440, loss of rhamnosyl, glucosyl and xylosyl groups). This corresponded to the fragmentation of cyanidin-3-xylosylrutinoside, which was previously detected in redcurrants (Oydvin, 1974; Goiffon et al., 1999; Maatta *et al.*, 2003).

Peak 6 (t_R – 31.4 min, λ_{max} – 355nm) had a $[M-H]^-$ at *m/z* 625 which with the loss of 308 *amu* yielded an MS^2 fragment corresponding to myricetin at *m/z* 317. These spectra indicated the presence of a myricetin rutinoside conjugate.

Peak 7 (t_R – 37.2, λ_{max} – 330 nm) yielded a $[M-H]^-$ at *m/z* 463 and MS^2 fragment at 317 ($[M-H]^-$-146, loss of a rhamnosyl moiety). It was, therefore, tentatively identified as a myricetin rhamnoside.

Peak 8 (t_R – 38.1 min, λ_{max} – 355 nm) was identified as quercetin-3-rutinoside. It yielded a $[M-H]^-$ at *m/z* 609, which with neutral loss of 308 *amu* yielded a MS^2 fragment corresponding to quercetin at *m/z* 301. The identification was confirmed by co-elution with a standard.

Peak 9 (t_R –39.6 min, λ_{max} – 355 nm) produced a typical quercetin glucoside mass spectrum ($[M-H]^-$ at *m/z* 463, MS^2 at *m/z* 301 and MS^3 at *m/z* 271, 255 and main ion at *m/z* 179). Co-chromatography established the presence of quercetin-3-glucoside.

Peak 10 (t_R – 43.4 min, λ_{max} 345 nm) had a $[M-H]^-$ at *m/z* 505 and MS^2 fragments at *m/z* 463 ($[M-H]^-$-42, loss of a malonyl-COOH group) and *m/z* 301 (quercetin, $[M-H]^-$ 204). As in peak 14 in blackcurrants (see Table 4.3), the 204 amu loss corresponds to the loss of hexose and malonyl groups, which in negative ion mode lost the 45 *amu* carboxylic function from the pseudomolecular ion. This is indicative of the presence of a quercetin malonylhexose conjugate. The presenece of such compound as previously been reported in redcurrants by Maatta *et al* (2003).

Peak 11 (t_R – 44.4 min, λ_{max} - 345 nm) yielded a $[M-H]^-$ at *m/z* 593 and an MS^2 fragment at *m/z* 285. The *m/z* 285 ion (kaempferol) was produced by a 308 *amu* cleavage, which corresponded to the loss of a rutinose unit. Peak 10 was therefore partially identified as a kaempferol rutinoside.

Peak 12 (t_R – 46.2 min, λ_{max} - 340 nm) had a $[M-H]^-$ at *m/z* 447 which with a loss of 162 *amu* (cleavage of hexose) produced a MS^2 fragment at *m/z* 285 (kaempferol). Co-elution with a standard established that this compound was kaempferol-3-glucoside.

4.1.3. Quantification of berry phenolics and their respective antioxidant capacity.

After identification of the phenolics in the berry extract by HPLC-diode array and MS^n detection, the extracts were further analysed by HPLC with a post-column $ABTS^{+\cdot}$ antioxidant detection system. Quantification of each compound and assessment of their individual antioxidant potential was carried out using this HPLC system. Figures 4.1 to 4.9 show the HPLC profiles of berries, the antioxidant contribution associated with each compound indicated by the peak recorded at 720 nm. This analysis enabled the antioxidant capacity of individual phenolic compounds to be determined. Antioxidant results are expressed as Trolox equivalents. Hydroxycinnamates (caffeic acid, 4-

hydroxybenzoic and *p*-coumaric conjugates) are expressed as 4-hydroxybenzoic acid equivalents; flavanols and their polymers in (+)-catechin equivalents, anthocyanin conjugates in cyanidin-3-glucoside equivalents, ellagic acid conjugates (except ellagitannins) in ellagic acid equivalents, ellagitannins in gallic acid equivalents, quercetin conjugates in quercetin-3-glucoside equivalents, kaempferol conjugates in kaempferol-3-glucoside equivalents and myricetin conjugates in myricetin equivalents. For each berry the contribution of individual components to the overall antioxidant activity was calculated as a percentage (% AOX) and reported in tables 4.18 to 4.24.

Note. The quantification in nmol/g of ellagitannins as well as procyanidins appear to be very high but this is due to the fact that these polymers are expressed as gallic acid or catechin equivalents, the molecular weights of which are very low compared to those of the polymers. In this way, the quantities did not correspond to the real number of molecules present in our extracts. However, no standards were available to allow more accurate estimate.

4.1.4. Discussion

Anthocyanin content

With the exception of raspberries, anthocyanins are the main phenolic compounds present in berries, where they are responsible for the red-purple colour. The nature of the anthocyanins and linked sugars varies among the different kinds of berries as reported in earlier studies (Goiffon *et al.*, 1991 and 1999, Nyman *et al.*, 2001, Dugo *et al.*, 2001). The identifications of individual anthocyanins in the present study was based using retention time data, absorbance at 520 nm, and positive ion MS data. Blackcurrants, strawberries, blackberries, cranberries and blueberries contain mainly monoglycosylated or acetylglucoside forms, whereas in redcurrants and raspberries the anthocyanins are linked to di-glycosides and even tri-glycosides.

With regard to the type of anthocyanins, redcurrants and blackberries contain only cyanidin conjugates (198 μg/g and 1557 μg/g respectively), strawberries only pelargonidin conjugates (253 μg/g) and raspberries mainly cyanidin conjugates (350 μg/g) as well as smaller amounts of pelargonidin derivatives (18 μg/g). In cranberries only peonidin (203 μg/g), cyanidin (142 μg/g) and malvidin (15 μg/g) conjugates were detected, whereas blueberries and blackcurrants contained delphinidin, cyanidin, petunidin, peonidin and malvidin linked to different hexose moieties (total content 2115

μg/g for blueberries and 3137 μg/g for blackcurrants), which were not always well separated in the HPLC. In strawberries the main anthocyanin was pelargonidin-3-glucoside, however, in keeping with the data of Tamura *et al.* (1995), the MS data also indicated the presence of pelargonidin-malonylglucoside. No trace was found of pelargonidin-3-arabinoside and cyanidin-3-glucoside previously reported to be present in strawberry by Goiffon *et al.* (1990, 1999). In cranberries we found mainly cyanidin-3-galactoside, peonidin-3-galactoside, cyanidin-3-arabinoside and peonidin-3-arabinoside, in agreement with Prior *et al.* (2001), as well as smaller amounts of malvidin-3-glucoside and malvidin-3-arabinoside, which were not previously detected. In blueberries, in agreement with Prior *et al.* (2001), 14 different anthocyanins were identified, including some acylated anthocyanins. In blackcurrants, in addition to the four major anthocyanins delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside previously detected by Goiffon *et al.* (1991, 1999) and Maatta *et al.* (2003), we also found peonidin-3-*O*-rutinoside, as did Froytlog *et al.* (1998). In agreement with Slimestad *et al.* (2002), pelargonidin-3-rutinoside, peonidin-3-glucoside, cyanidin-3-arabinoside, malvidin-3-glucoside, delphinidin-3-*O*-(6″-coumaroylglucoside). A range of other anthocyanins including delphinidin-3-galactoside, delphinidin-3-arabinoside, peonidin-3-galactoside, malvidin-3-galactoside and peonidin-3-arabinoside were also detected in the present study. These differences could be due to the variety analysed (unknown in the present study). In blackberries, the main anthocyanin was cyanidin-3-glucoside, as reported by Goiffon *et al.* (1991) and Dugo *et al.* (2001); traces of cyanidin-3-rutinoside as well as cyanidin malonylglucoside were also identified. In redcurrants, cyanidin-3-sambubioside, cyanidin-3-rutinoside and cyanidin-3-xylosylrutinoside were the main anthocyanins, in agreement with Goiffon *et al.* (1991, 1999), with a total anthocyanin content of 198 μg/g very similar to the 177 μg/g reported by Maatta *et al.* (2003). In raspberries, nine different anthocyanins were present, the major components being cyanidin-3-sophoroside, cyanidin-3-(2^G-glucosylrutinoside) and cyanidin-3-glucoside as reported by Mullen *et al.* (2003).

Flavonol content

The flavonol content of berries has been investigated in many studies (Hakkinen *et al.*, 1999 and 2000, Justesen *et al.*, 1998, Sellapan *et al.*, 2002). In these investigations analyses were carried after acid hydrolysis of the extracts, providing information only on the aglycone forms (quercetin, kaempferol and myricetin). More recently Maatta *et al.*

(2003) and Vvedenskaya *et al.* (2004) used MS and NMR techniques to identify flavonols in blackcurrants and cranberries respectively.

In the present study all berries, with the exception of strawberries where kaempferol is the principal form, contained quercetin conjugates as the main flavonols present (5 μg/g in strawberries and up to 300 μg/g in blueberries). Myricetin and/or kaempferol conjugates were also detected in most of the berries. Myricetin has been reported to be the main flavonol present in blackcurrants (Hakkinen *et al.*, 1999) followed by quercetin. More recently Maatta *et al.* (2003) reported the reverse of this with 47 μg/g and 40 μg/g of quercetin and myricetin conjugates respectively. In the present study the levels of myricetin were quite similar with 54 μg/g (due mainly to the presence of myricetin-3-rutinoside and myricetin-glucuronide), but quercetin conjugates were found in higher levels (102 μg/g).

Total flavonols levels were in the range 9 μg/g in raspberries and 171 μg/g in blackcurrants. These values are in accordance with the data of Hakkinen *et al.* (2000), who reported a flavonol content of 17 μg/g in strawberries up to 157 μg/g in blackcurrants). Flavonols are mainly present as hexose, glucuronide, rutinoside, rhamnoside or pentose conjugates.

Ellagic acid conjugates and ellagitannins content

High levels of ellagitannins (988 μg/g of gallic acid equivalents) were identified in raspberries including sanguiin H-10, sanguiin H-6 and lambertianin C, as reported by Mullen *et al.* (2003). Ellagitannins were also found in strawberries (75 μg/g of a dimeric ellagitannin tentatively identified as a sanguiin like compound) and in blackberries (lambertianin C, sanguiin H-6, H-10 and another dimer with a total content of 650 μg/g). Raspberries contained 13 μg/g of ellagic acid free or linked to pentose and acetylxyloside as reported by Mullen *et al.* (2003). Similar compounds were found in strawberries (with the presence of 6.0 μg/g of ellagic acid-rhamnose) and in blackberries (5.4 μg/g of ellagic acid rutinoside hexose conjugate). Hakkinen *et al.* (1999) reported 1500 μg/g ellagic acid in raspberries, 630 μg/g in strawberries (dry weight), whereas Sellapan *et al.* (2002) found 300 μg/g fresh weight in blackberries after acid hydrolysis of the extracts. As previously noted by Mullen *et al.* (2002a), ellagitannins are the primary source of ellagic acid released by acid hydrolysis of raspberry extracts. This observation may also apply to strawberries and blackberries. No ellagic acid conjugates were found in the other berry

extracts in this study, although Daniel *et al.* (1989) reported 120 μg/g (dry weight) of ellagic acid after acid hydrolysis of cranberries.

Flavan-3-ols

Few studies report flavan-3-ol levels in berries. Kahkonen *et al.* (2001) reported a very low level of flavan-3-ols and their polymer procyanidins in strawberries and raspberries (81-100 μg/g and 30-40 μg/g dry weight respectively of catechin equivalents), 680 μg/g in redcurrants, 1140-2080 μg/g in blackcurrants and a much higher level (4230 μg/g) in cranberries. In the present study, traces of catechin were found in both raspberries and blackberries, whereas strawberries contained catechin dimers and trimers (161 μg/g of catechin equivalents in total). In cranberries we found 149 μg/g of (-)-epicatechin and a higher amount (1405 μg/g) of procyanidins (dimer, hexamer and other procyanidin polymers. These data were in agreement with Prior *et al.* (2001) who also reported the presence of different procyanidins in cranberries (monomers to heptamers doubly or singly linked).

Note. Procyanidin polymers could have been underestimated, in particular in redcurrants, as these compounds have been shown to be retained in the cell wall (see chapter 5) and be released only after acid hydrolysis. Such investigations were not carried out in this work.

Phenolic acid conjugates

Some caffeic acid conjugates were identified using MS data in cranberries, redcurrants and blackcurrants. *p*-Coumaric acid hexose conjugates were present in cranberries and strawberries, caffeoylquinic acids in cranberries, blueberries and blackcurrants as well as 4-hydroxybenzoic acid hexose conjugates in redcurrants and feruloylquinic acid in blueberries. With the exception of blueberries, which contained 390 μg/g of caffeoylquinic acid, the levels of these phenolic acid conjugates were very low in berries. Schuster *et al.* (1985) reported a wider distribution of phenolic acid glucosides in berries. Hydroxycinnamoyl hexose conjugates were found in all berries, in varying amount, mainly as *p*-hydroxycinnamic acids, whereas for caffeic acid quinates esters were predominant. Levels reported were quite often under 10 μg/g (lower than our level of UV detection. Using MS data we could have investigated and quantified these minor components, however the aim was to identify and quantify the main antioxidant contributors in berries rather than determine all the phenolic compounds present.

On-line antioxidant analyses

In berries, with the exception of raspberries, anthocyanins were the most abundant flavonoids, but the antioxidant profile showed that anthocyanins were not always the major source of antioxidants. Indeed, only in blackcurrants and blueberries were anthocyanins the main antioxidant contributors (with 81 % and 70 % respectively). For raspberries, blackberries and strawberries, ellagitannins were the main contributors to the antioxidant activity (with 69, 41 and 21 % respectively), whereas in redcurrants the antioxidant activity was mainly related to vitamin C content (56 %) and to anthocyanins (22 %).

The contribution of vitamin C to the total antioxidant capacity varied between 0% in blueberries to 56.4% in redcurrants even if the quantity present (174 μg/g) was not high compared to the other berries. Conversly blackcurrants contained the highest level of vitamin C (508 μg/g) but it contributed only 10.7% of antioxidant potential.

Ellagitannins are very powerful antioxidants, contributing 21%, 37% and 70% of the overall antioxidant potential in strawberry, raspberry and blackberry respectively. Previous work using fractionation by preparative HPLC revealed that sanguiin H-6 was a major contributor to the antioxidant capacity of raspberries (Mullen *et al.*, 2002).

Flavonols were very poor contributors to the antioxidant capacity ranging from 0 % in raspberries and strawberries to 5.5% in blueberries. This phenomenon is probably due to two factors. First flavonols linked to sugars have lower antioxidant capacity compared to their aglycones; Plumb *et al.* (1997) reported a TEAC value of 1.45 for quercetin-3-*O*-sophoroside much lower than the 4.43 reported for quercetin. Secondly levels of flavonols present in berries were low.

The phenolic acids did not contribute significantly to the antioxidant potential of berries, as they were present in very small amounts. Nonetheless, their potential antioxidant activity was relatively high.

Flavan-3-ols were also minor contributor to the total antioxidant activity, except in cranberries. Indeed for cranberries, the situation is more complex as flavan-3-ols co-elute with flavonols and anthocyanins. On their own, flavan-3-ols contribute up to 15 % of the antioxidant capacity, and could be as high as 49 % if we include peak 6, 9, 12 and 13, where flavan-3-ols co-elute with other flavonoids. This is in agreement with Prior *et*

al. (2001), who reported that the procyanidin fraction of cranberries accounts for up to 54% of the total antioxidant activity (measured by ORAC).

4.2. Fruits and vegetables

A range of potentially high antioxidant containing fruits and vegetables comprising kiwi, clementine, cherry tomato, curly kale, purple broccoli, broccoli, red, yellow and green peppers (chosen using Borges *et al.* unpublished data) were purchased in a local supermarket in Glasgow.

4.2.1 Antioxidant activity, total phenol and vitamin C content

4.2.1.1. Antioxidant activity and total phenol content

The total antioxidant activity (measured by FRAP in μM/g of Fe^{2+} equivalent) as well as the total phenol content (expressed as mg/g of gallic acid equivalent) are presented in Figure 4.2.

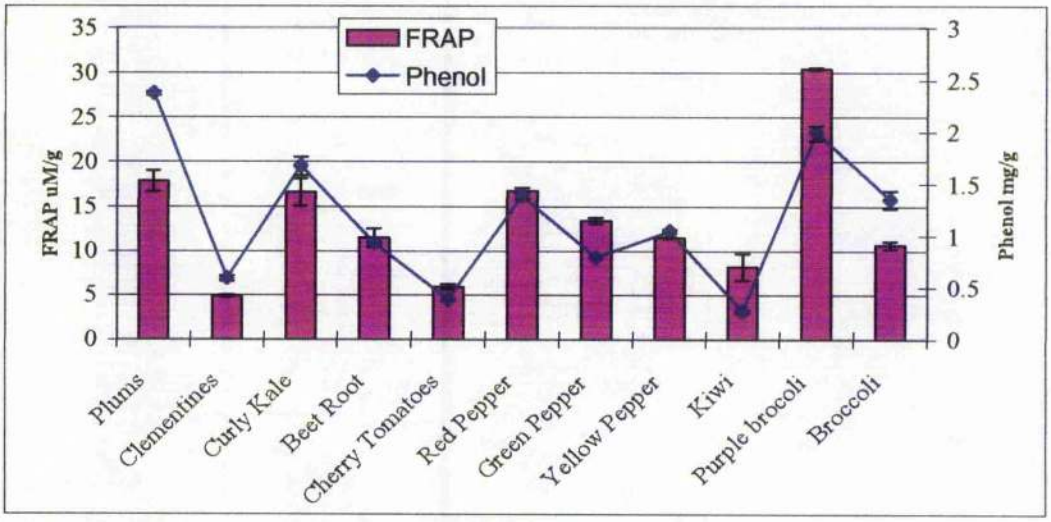


Figure 4.2. Antioxidant activity and total phenol content of fruits and vegetables

There were large differences in the antioxidant capacities of the different fruits and vegetables analysed. Purple broccoli, followed by plums, curly kale and red pepper had higher antioxidant properties (with 30.4 ± 0.1, 17.8 ± 1.2, 16.6 ± 1.5 and 16.8 ± 0.3 μM/g Fe^{2+} equivalent). At the other end of the scale, clementines and tomatoes were less powerful with 4.9 ± 0.1 and 5.9 ± 0.3 μM/g Fe^{2+} equivalents respectively. Broccoli

possessed much less antioxidant activity than purple broccoli and curly kale. These values were in the same range as earlier data. Indeed, using FRAP assay, Pellegrini *et al.* (2003) have reported 5.1 μM/g in tomatoe, 7.4 μmol/g in kiwi, 8.9 μM/g in clementines, 11.7 μM/g in broccoli and 20.9 μM/g in red pepper. Ou *et al.* (2002 and 2003) reported that red, green and yellow peppers were more potent antioxidants (with 185 ± 49, 157 ± 58 and 457 ± 58 μM/g dry weight of Trolox equivalents respectively) compared to broccoli and tomato (41 ± 11 and 56 ± 8). These values presented the same trend as obtained in present study but data were expressed in Trolox rather than Fe^{2+} equivalent.

4.2.1.2. Vitamin C content

The vitamin C content was analysed by gradient HPLC with detection at 262 nm as described by Ross (1994). Results are included in tables 4.25 to 4.34. Plums contain low levels of vitamin C (3 μg/g) as compared to kiwi (858 μg/g) or peppers (1122, 1137 and 1668 μg/g for yellow, green and red pepper respectively).

4.2.2. Identification of polyphenols by MS analysis

Methanolic extracts of fruit and vegetables were analysed using the LC-MS system described in section 4.1.2, with a 60 min, 5-30% gradient of acetonitrile in 1% formic acid. All samples were analysed with the mass spectrometer operating in negative ionisation mode; plums and purple broccoli were also subjected to analysis in positive ion mode, as they contained anthocyanins. In addition to the full scan analysis, in source fragmentation was carried on to confirm identification.

4.2.2.1. Curly Kale

The HPLC traces obtained at 520, 365 and 280 nm as well as MS^n analysis facilitated the detection of 25 phenolic compounds (24 peaks) (Fig. 4.10). As some of the flavonols were highly glucosylated and acylated, the molecular weights were high, therefore in source fragmentation (ISF) analysis was carried out to obtain further fragmentation and identify the aglycons present. Curly kale was also acid hydrolysed to confirm the presence of quercetin and kaempferol (rather than other aglycones having the same molecular weight). Where reference compounds were not available identifications were facilitated by previous data with cauliflower extracts on the MS fragmentation patterns of flavonols and the *m/z* losses associated with cleavage of various sugars and other

substituent groups (Llorach *et al.*, 2003). These authors reported general rules concerning the MS fragmentation and the chromatographic behaviour of the highly glycosylated and acylated flavonols, which can be summarised as follows.

(i) the introduction of a glucose on the hydroxyl at C-7 dramatically reduces the retention time, whereas adding a second one increases the retention time.

(ii) increased glycosylation at C-3 lead to a shortening of retention times.

(iii) the MS fragmentation of these compounds showed a common rule: the initial loss was the sugar at C-7, followed by the acyl residues and then the loss of C-3 sugars.

(iv) the acylation with hydroycinnamic acid increased the retention time, and reduces the UV absorption.

Identification of highly glycosylated and acylated flavonols was even more difficult as a loss of 162 *amu* could either correspond to an hexose or a caffeoyl moiety, and similarly a loss of 176 *amu* could be a glucuronide or a feruloyl group. In cauliflower, Llorach *et al.* (2003) reported glucose was the only sugar present. By consequent, a loss of a 176 amu was always associated to a loss of feruloyl moiety for curly kale and broccoli in the present study.

The data of Price *et al.* (1997) and Vallejo *et al.* (2003) also aided the MS-based identifications of hydroxycinnamoyl derivatives.

The identification of phenolic compounds in HPLC peaks 1-24 in Figure 4.10, based on MS^2 and MS^3 data, as well as λ_{max}, are summarised below and presented in Table 4.8.

Peak 1 (t_R – 11.2 min, λ_{max} – 325 nm) contained two different compounds. One had a $[M-H]^-$ at *m/z* 787, which with neutral loss of three successive 162 *amu* units, yielded a MS^2 fragment corresponding to quercetin at *m/z* 301. This compound was tentatively identified as a quercetin-trihexose conjugate. The second had a $[M-H]^-$ at *m/z* 353, which fragmented to give a main fragment at *m/z* 191 and a second major ion at *m/z* 179, characteristic of 3-caffeoylquinic acid (Clifford *et al.*, 2003).

Peak 2 (t_R – 13.2 min, λ_{max} – 353 nm) showed a $[M-H]^-$ at *m/z* 787, consistent with quercetin trihexose conjugate. It yielded MS^2 fragments at *m/z* 625 ($[M-H]^-$-162, loss of an hexose unit), *m/z* 463 ($[M-H]^-$-324, loss of an additional hexose) and *m/z* 301 (quercetin, $[M-H]^-$-486 corresponding to the successive loss of three hexose moieties). MS properties, elution time and UV absorption matched those of quercetin-3-diglucoside-7-glucoside, identified previously by Llorach *et al.* (2003) in cauliflower.

Peak 3 (t_R –14.5 min, λ_{max} – 320 nm) had a $[M-H]^-$ at *m/z* 807, which on MS^2 produced fragments at *m/z* 609 ($[M-H]^-$-198) and *m/z* 447 ($[M-H]^-$-360, corresponding to the loss of a 198 *amu* and a hexose unit). Further fragmentation of pseudomolecular ion at *m/z* 609 produced an ion at *m/z* 285, characteristic of kaempferol. This compound was identified as a kaempferol conjugate, containing 2 hexose moieties and an unidentified group, which produces a neutral loss of 198 *amu*.

Peak 4 (t_R –15.3 min, λ_{max} – 345 nm) had a $[M-H]^-$ at *m/z* 771 which yielded MS^2 and MS^3 fragment ions at *m/z* 609, ($[M-H]^-$ 162, loss of a hexose unit), *m/z* 429 and *m/z* 285 (kaempferol, ($[M-H]^-$-486, successive loss of three hexose moieties). These properties matched those of kaempferol-3-diglucoside-7-glucoside, which have been identified in cauliflower (Llorach *et al.*, 2003).

Peak 5 (t_R –16.1 min, λ_{max} – 335 nm) produced a $[M-H]^-$ at *m/z* 979 which fragmented to produce MS^2 ions at *m/z* 817($[M-H]^-$-162, loss of a hexose unit), *m/z* 787 ($[M-H]^-$-192), and *m/z* 625 ($[M-H]^-$-354, loss of a hexose and a 192 *amu* unit). The MS^3 analysis of ion at *m/z* 787 showed the sequential loss of two hexoses followed by the loss of another hexose to obtain the quercetin aglycone at *m/z* 301. This peak was therefore identified as a quercetin conjugate with three hexoses and an unknown group, which produces a neutral loss of 192 *amu*.

Peak 6 (t_R –17.9 min, λ_{max} – 330 nm) had a $[M-H]^-$ at *m/z* 963 which with neutral loss of a 162 *amu* yielded a MS^2 at *m/z* 801. MS^3 analysis of this ion showed the sequential loss of a 192 *amu* unit, followed by the loss of two hexose units to obtain the kaempferol aglygone. This compound was identified as a kaempferol-dihexoside conjugate, again with an unknown unit producing a neutral loss of 192 *amu*. Similar compounds have been reported previously in cauliflower (Llorach *et al.*, 2003).

Peak 7 (t_R 18.2 min, λ_{max} 335 nm) also had a $[M-H]^-$ at *m/z* 963, which yielded on MS^2 fragments at *m/z* 801 ($[M-H]^-$-162, loss of a hexose moiety), *m/z* 787 ($[M-H]^-$-176, loss of a feruloyl unit), *m/z* 625 ($[M-H]^-$-338, loss of both a feruloyl and an hexosyl unit) and *m/z* 445. This fragmentation pattern matched that of kaempferol-3-feruloylglucoside-7-glucoside (at *m/z* values 16 *amu* higher, corresponding to the additional methyl group present in quercetin) identified previously in cauliflower by Llorach *et al.* (2003). Presence of the quercetin aglycone (at *m/z* 301) was confirmed by ISF analysis of curly kale. Peak 7 was therefore tentatively identified as quercetin-3-feruloyl-diglucoside-7-glucoside.

Peak 8 (t_R –19.2 min, λ_{max} – 340 nm) was a minor flavonol. It had $[M-H]^-$ at *m/z* 1155, which on MS^2 yielded fragments at *m/z* 993 ($[M-H]^-$-162, cleavage of a hexose moiety), *m/z* 949 ($[M-H]^-$-206, loss of a sinapoyl unit), *m/z* 787 ($[M-H]^-$-368, loss of a sinapoyl and a hexose unit). This compound was identified as quercetin sinapoyl-tetraglucoside.

Peak 9 (t_R –19.5 min, λ_{max} – 340 nm) had a $[M-H]^-$ at *m/z* 1317 consistent with a quercetin pentaglucoside acylated with a sinapic residue. This hypothesis was confirmed by MS fragmentation pattern. Indeed this ion produced on MS^2 fragments at *m/z* 1111 ($[M-H]^-$-206, loss of a sinapoyl), *m/z* 993 ($[M-H]^-$-324, loss of two hexose units), *m/z* 787 ($[M-H]^-$-530, loss of two hexose and a sinapoyl moiety), and *m/z* 463 ($[M-H]^-$ 854, corresponding to the additional cleavage of two hexose units).

Peak 10 (t_R –19.9 min, λ_{max} – 340 nm) produced a $[M-H]^-$ at *m/z* 1177, which yielded on MS^2 fragments at *m/z* 853 ($[M-H]^-$-324, loss of two hexose unit), *m/z* 647 ($[M-H]^-$-530, cleavage of an additional sinapoyl), and *m/z* 449 by cleavage of an additional fragment at 198 *amu*. This compound was therefore identified as a kaempferol conjugate, kaempferol triglucoside, acylated with a sinapoyl and another unknown unit at *m/z* 198.

Peak 11 (t_R –20.2 min, λ_{max} – 340 nm) is the main flavonol present in curly kale. It produced a major $[M-H]^-$ ion at *m/z* 993, which on MS^2 yielded a range of fragments ion at *m/z* 831, 787, 625 and 445. The MS^2 ions obtained correspond to the loss of a hexose or a sinapoyl moiety (*m/z* 831 and 787 respectively), and the loss of a sinapoyl and two hexose moieties (*m/z* 625). On the basis of MS data, peak 11 was tentatively identified as quercetin-3-sinapoyldiglucoside-7-glucoside, which has been previously identified in cauliflower by Llorach *et al.* (2003).

Peak 12 (t_R –20.7 min, λ_{max} – 340 nm) produced a $[M-H]^-$ ion at *m/z* 963 which fragmented on MS^2 to produce fragments at *m/z* 801 ($[M-H]^-$ -162, cleavage of a hexose moiety), *m/z* 787 ($[M-H]^-$-176, loss of a feruloyl group) and *m/z* 625 ($[M-H]^-$-338, loss of feruloyl and glucosyl unit). This compound was, therefore, a quercetin-feruloyl-triglucoside.

Peak 13 (t_R –21.8 min, λ_{max} – 330 nm) showed that the $[M-H]^-$ pseudomolecular ion at *m/z* 1139 lost a glucose to produce a fragment at *m/z* 977. MS^2 analysis of this daughter ion showed the sequential loss of a sinapic acid (*m/z* 771) followed by the loss of another hexose moiety (*m/z* 609). When this peak was analysed by ISF, it showed the

presence of the kaempferol aglycone ([M-H]$^-$ at *m/z* 285, producing fragment at *m/z* 255 and *m/z* 179). Peak 13 is therefore a kaempferol-sinapoyl-pentaglucoside, possibly kaempferol-3-sinapoyltriglucoside-7-glucoside as MS and UV properties matched those of this compound identified previously in cauliflower (Llorach *et al.*, 2003).

Peak 14 (t_R –22.4 min, λ_{max} – 330 nm) had a [M-H]$^-$ ion at *m/z* 977, which on MS^2 produced a fragment at *m/z* 815 ([M-H]$^-$-162 corresponding to the loss of a glucose), *m/z* 609 ([M-H]$^-$-368, loss of an additional sinapoyl residue) and *m/z* 447 (cleavage of another glucosyl moiety). This compound was identified as a kaempferol-sinapoyl-triglucoside.

Peak 15 (t_R –27.5 min, λ_{max} – 335 nm) had a [M-H]$^-$ ion at *m/z* 1139, producing on MS^2 the same fragments as observed for peak 14 at *m/z* 815 and 609 (corresponding respectively to the loss of two hexose units, and an additional sinapoyl fragment). MS^3 of the ion at *m/z* 609 produced an ion at *m/z* 447, corresponding to the loss of a 162 *amu,* as well as the kaempferol aglycone at *m/z* 285 (after the sequential loss of two hexose units). This compound was identified as a kaempferol sinapoyl-tetraglucoside.

Peak 16 (t_R –31.6 min, λ_{max} – 345 nm) had a [M-H]$^-$ ion at *m/z* 831 consistent with a quercetin-sinapoyl-diglucoside. The MS^2 released ions at *m/z* 625 (by cleavage of a sinapoyl 206 *amu*), 445 and 301 (quercetin, [M-H]$^-$-531, cleavage of two additional hexose moieties). This compound was tentatively identified as quercetin-3-sinapoyldiglucoside, as its fragmentation pattern matched, at *m/z* 16 *amu* higher, those of kaempferol-3-sinapoyldiglucoside, previously reported in cauliflower by Llorach *et al.* (2003).

Peak 17 (t_R –33.1 min, λ_{max} – 330 nm) was quercetin-3-rutinoside, having a [M-H]$^-$ ion at *m/z* 609, which with neutral loss of 308 *amu,* corresponding in this instance to the loss of a glucosyl and a rhamnosyl unit, yielded an MS^2 fragment corresponding to quercetin at *m/z* 301. This was confirmed by co-elution with an authentic standard.

Peak 18 (t_R –36.0 min, λ_{max} – 335 nm) had a [M-H]$^-$ ion at *m/z* 1523, which on MS^2 produced ions at *m/z* 1199, 993 and 787 corresponding to the sequential loss of two hexose moieties, followed by a sinapoyl unit and then an additional sinapoyl. This compound was therefore a quercetin disinapoylpentaglucoside, possibly quercetin-3-disinapoyltriglucoside-7-diglucoside, as again its properties (at 16 *amu* higher) matched those of the kaempferol-3-disinapoyl-7-diglucoside identified previously in cauliflower (Llorach *et al.*, 2003).

Peak 19 (t_R –37.3 min, λ_{max} – 335 nm) had a $[M-H]^-$ ion at *m/z* 1361, consistent with a quercetin disinapoyl-tetraglucoside. MS^2 analysis are in agreement with this hypothesis as it showed fragments at *m/z* 1199 ($[M-H]^-$ -162, loss of a hexose unit), *m/z* 1155 ($[M-H]^-$-206 loss of a sinapoyl unit) and *m/z* 787 ($[M-H]^-$-574, loss of an hexose and two sinapoyl units).

Peak 20 (t_R –39.0 min, λ_{max} – 330 nm) produced a $[M-H]^-$ ion at *m/z* 1345, which produced on MS^2 fragments at *m/z* 1183 (cleavage of an hexosyl unit) and *m/z* 977 (loss of a sinapoyl fragment). This compound could be kaempferol-3-disinapoyltriglucoside-7-glucoside, the presence of which has been reported in cauliflower by Llorach *et al.* (2003), as elution time, fragmentation pattern are very similar.

Peak 21 (t_R –45.6 min, λ_{max} – 330 nm) showed a pseudomolecular ion at *m/z* 753 consistent with a disinapoylglucoside. It produced fragments on MS^2 at *m/z* 529 ($[M-H]^-$ -224, loss of a sinapic acid) and was identified as 1,2-disinapoyldiglucoside, a known constituent of broccoli and cauliflower (Price *et al.*, 1997; Vallejo *et al.*, 2003; Llorach *et al.*, 2003).

Peak 22 (t_R –47.3 min, λ_{max} – 330 nm) was similarly identified as 1-sinapoyl-2-feruloyldiglucoside as it had a $[M-H]^-$ ion at *m/z* 725, yielded MS^2 at *m/z* 501 by neutral loss of a 224 *amu*. Its presence has been reported in broccoli (Price *et al.*, 1997; Vallejo *et al.*, 2003) and cauliflower (Llorach *et al.*, 2003).

Peak 23 ($[t_R]$ –53.0 min, λ_{max} – 325 nm) had a $[M-H]^-$ ion at *m/z* 961, producing on MS^2 fragments at *m/z* 737 ($[M-H]^-$ -224, loss of a sinapic acid unit), *m/z* 531 and 325 by cleavage of one and two additional 206 *amu* sinapoyl residues respectively. It was therefore identified as 1,2,2'-trisinapoyldiglucoside. This was confirmed by previous work (Price *et al.*, 1997; Vallejo *et al.*, 2003; Llorach *et al.*, 2003).

Peak 24 (t_R –54.6 min, λ_{max} – 325 nm) had a $[M-H]^-$ ion at *m/z* 931, producing fragment ions at *m/z* 707 and 501, consistent with the presence of a diglucoside ester linked to both a ferulic acid and two sinapic acid units. Previous NMR data had identified the ester position and mode of linkage. Therefore, this compound was identified as 1,2'-disinapoyl-2-feruloyldiglucoside, a known constituent of cauliflower and broccoli (Price *et al.*, 1997; Vallejo *et al.*, 2003; Llorach *et al.*, 2003).

Note. The presence of highly glycosylated acylated quercetin and kaempferol was confirmed as acid hydrolysis of curly kale extracts produced high levels of free, monoglucoside or diglucoside quercetin and kaempferol, whereas peak 1-20 pratically

disappear (see Figure 4.20). Furthermore the only aglycone at *m/z* 301 and *m/z* 285 were positively identified as quercetin and kaempferol respectively.

4.2.2.2. Purple broccoli

Purple broccoli contained 43 different polyphenolic compounds, mainly present as highly glycosylated and acylated flavonols and anthocyanins, or hydroxycinnamate conjugates. ISF analysis was carried on to confirm the identity of the aglycones present in certain peaks. A purple broccoli extract was also acid hydrolysed, to confirm the presence of the aglycones quercetin, kaempferol, and cyanidin. Where reference compounds were not available identifications were facilitated by previous data on the MS fragmentation patterns of flavonols and the neutral losses associated with cleavage of various sugars and other substituent groups presented by Llorach *et al.* (2003) on cauliflower extracts (see rules summarised in section 4.2.2.1). Similarly, the data of Price *et al.* (1997) and Vallejo *et al.* (2003) aided the MS-based identifications of hydroxycinnamoyl derivatives. For the anthocyanins, previous work on red cabbage (Stintzing *et al.*, 2002; Giusti *et al.*, 1999; Degenhardt *et al.*, 2000; Dyrby *et al.*, 2001) helped the identification of the highly conjugated cyanidin derivatives. The identification of phenolic compounds in HPLC peaks 1-41 in Figure 4.11, based on MS^2 and MS^3 data, as well as λ_{max}, are summarised below and presented in Table 4.9.

Peak 1 (t_R –10.6 min, λ_{max} – 515 nm) was a minor anthocyanin. It had a $[M+H]^+$ ion at *m/z* 773, which produced on MS^2 fragments ion at *m/z* 611, 449 and 287 by the sequential loss of one, two and three glucose moieties respectively. It was, therefore, a cyanidin triglucoside. One possible candidate is cyanidin-3-diglucoside-5-glucoside, as this compound has been identified in red cabbage (Giusti *et al.*, 1999; Dyrby *et al.*, 2001).

Peak 2 (t_R –11.2 min, λ_{max} – 325 nm) had a $[M-H]^-$ ion at *m/z* 353 and on MS^2 produced two major ions at *m/z* 191 and 179. On basis of this MS data, this compound was identified as 3-caffeoylquinic acid (Clifford *et al.*, 2003).

Peak 3 and 13(t_R –12.9 and 18.5 min, λ_{max} – 355 nm) both had a $[M-H]^-$ ion at *m/z* 787, which on MS^2 yielded fragments at *m/z* 625 ($[M-H]^-$-162, loss of a hexose unit) and *m/z* 301 (quercetin, $[M-H]^-$-486, corresponding to the loss of three hexose moieties). Peak 13 had an additional ion fragment at *m/z* 463 ($[M-H]^-$-324, successive loss of two hexose groups). These compounds were both tentatively identified as quercetin-trihexosides.

Peaks 4, 5 and 14 (t_R –13.5, 14.3 and 19.0 min, λ_{max} – 355, 350 and 350 nm) had a pseudomolecular ion at *m/z* 949, consistent with a quercetin tetrahexoside. Peaks 4 and 14 yielded MS^2 fragment ions at *m/z* 625 and *m/z* 301, corresponding to the sequential loss of two dihexoside units. Peak 5 produced an additional MS^2 fragment at *m/z* 787 by the loss of a 162 *amu*, and MS^3 at *m/z* 625, 463 and 301 corresponding to the additional loss of one, two and three hexose units. These compounds were all identified as quercetin tetrahexoside, with the hexose moieties location at different poitions on the flavonol ring.

Peak 6 (t_R –14.8 min, λ_{max} – 320 nm) had a $[M-H]^-$ ion at *m/z* 807, which yielded on MS^2 fragment ions at *m/z* 609 ($[M-H]^-$ -198), *m/z* 447 ($[M-H]^-$-162, loss of a hexose unit) and *m/z* 285 (kaempferol, $[M-H]^-$-522, loss of two hexose units and a 198 *amu* group). This compound was identified as a kaempferol conjugate containing two hexose moieties and an unknown group which produces a neutral loss of 198 *amu*.

Peak 7 (t_R –15.6 min, λ_{max} – 325 nm) had a $[M-H]^-$ ion at *m/z* 816 and MS^2 fragments at *m/z* 655 ($[M-H]^-$-162, loss of a hexosyl unit), *m/z* 609 ($[M-H]^-$-206, loss of a sinapoyl moiety) and *m/z* 285 ($[M-H]^-$-532, corresponding to the sequential loss of a sinapoyl and two hexose units). MS^3 of this ion produced fragments at *m/z* 255 confirming the presence of kaempferol. Peak 7 was identified as a kaempferol-sinapoyl-diglucoside.

Peak 8 (t_R –16.2 min, λ_{max} – 345 nm) produced a $[M-H]^-$ at *m/z* 969, which yielded on MS^2 fragments at *m/z* 609 ($[M-H]^-$-360, loss of a hexose unit and an unknown 198 *amu* unit). This ion produced on MS^3 fragments at *m/z* 447 and 285, by sequential cleavage of 2 hexose units. This compound was therefore identified as a kaempferol conjugate (kaempferol-triglucoside, acylated with an unknown unit which on MS cleaves with a loss of 198 *amu*.).

Peak 9 (t_R –16.7 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 1111, consistent with a quercetin pentaglucoside. It produced on MS^2 fragments at *m/z* 949, 787 and 625, corresponding to the successive cleavage of one, two and three hexose units. The MS^3 analysis of the 949 *m/z* ion showed the sequential loss of one, two and three hexose units to obtain the quercetin aglycone. Peak 9 was therefore identified as a quercetin-pentahexoside.

Peak 10 (t_R –17.1 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 1273, which yielded on MS^2 fragments at *m/z* 1111, 949, 787 and 625 by the sequential loss of one, two, three and four neutral 162 *amu*. The 162 *amu* could correspond to either hexose unit or

caffeoyl moiety. This compound could therefore be a quercetin-hexa-hexoside or a quercetin pentahexoside acylated with caffeic acid.

Peak 11 (t_R –18.2 min, λ_{max} – 325 nm) had a $[M-H]^-$ at *m/z* 1287, which produced on MS^2 a sequence of fragments at *m/z* 963 ($[M-H]^-$-324, corresponding to the cleavage of two hexose units), *m/z* 771 ($[M-H]^-$-516, cleavage of an additional unknown 192 *amu* fragment), *m/z* 753 and 429. Peak 11 was identified as a kaempferol conjugate (kaempferol-pentahexoside, acylated with an unknown unit which produces a neutral loss of 192 *amu*).

Peak 12 (t_R –18.3 min, λ_{max} – 335 nm) produced an ion at *m/z* 1125, which yielded MS^2 fragments at *m/z* 801 and *m/z* 609, which in this instance corresponded to the loss of two hexose units, followed by the loss of an unknown fragment 192 *amu*. The MS^3 analysis of the ion at *m/z* 801 showed fragments at *m/z* 609 and *m/z* 285 by the cleavage of a 192 *amu* fragment and two other hexose moieties. Peak 13 was another kaempferol conjugate, containing four hexoses and an unknown acyl, which produces a neutral loss of 192 *amu*.

Peak 15 (t_R –19.2 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 1095, consistent with a kaempferol pentahexoside. The MS^2 analysis showed the sequential loss of one, two and three hexose moieties. ISF analysis showed a pseudomolecular ion at *m/z* 285, which yielded on MS^2 fragment ions at *m/z* 255, consistent with the fragmentation pattern of kaempferol. This compound was therefore identified as a kaempferol-pentahexoside.

Peak 16 (t_R –20.3 min, λ_{max} – 340 nm) is a major flavonol present in purple broccoli. It had a $[M-H]^-$ at *m/z* 1287, which produced on MS^2 ions at *m/z* 1111 ($[M-H]^-$ - 176, corresponding to the cleavage of a feruloyl group), *m/z* 963 ($[M-H]^-$ -324, loss of two hexose moieties) and *m/z* 787 ($[M-H]^-$-500, cleavage of a feruloyl and two hexose units). ISF analysis showed the presence of ions at *m/z* 463, 301, 271 and 255, confirming the presence of quercetin. Peak 16 was identified as a quercetin-feruloyl-pentaglucoside.

Peak 17 (t_R –21.2 min, λ_{max} – 330 nm) produced a $[M-H]^-$ at *m/z* 1301, which produced on MS^2 a sequence of fragments by the sequential loss of two glucose moieties (*m/z* 977), followed by an additional sinapoyl unit (*m/z* 771). ISF analysis showed a pseudomolecular ion at *m/z* 285, producing on MS^2 a fragment at *m/z* 255, characteristic of a kaempferol aglycone. This compound was identified as a kaempferol-sinapoyl-pentaglucoside.

Peak 18 (t_R –21.7 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 1139, which with neutral loss of a 162 *amu* hexosyl unit yielded on MS^2 a fragment at *m/z* 977. MS^3 analysis of this ion showed the seqential loss of a sinapoyl unit (*m/z* 771), followed by the loss of three other hexose moieties, to produce a kaempferol aglycone at *m/z* 285. This compound was identified as a kaempferol-sinapoyl-tetraglucoside.

Peak 19 (t_R –21.9 min, λ_{max} – 340 nm) produced a $[M-H]^-$ at *m/z* 933, consistent with a kaempferol-tetrahexoside. It yielded on MS^2 fragment at *m/z* 609 and 285, by the cleavage of two and four hexose moieties respectively. Peak 19 was identified as a kaempferol tetrahexoside, possibly with two disaccharides located at different positions on the kaempferol skeleton.

Peak 20 (t_R –22.4 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 1271 and MS^2 fragments at *m/z* 1109 ($[M-H]^-$-162, loss of a hexose unit), *m/z* 947 ($[M-H]^-$-324, cleavage of an additional hexose group), *m/z* 771 ($[M-H]^-$-500, cleavage of an additional feruloyl unit), and *m/z* 609 ($[M-H]^-$-662, cleavage of another hexose unit). ISF analysis showed the presence of ions at *m/z* 285 and 255, and confirmed the presence of a kaempferol conjugate. Peak 20 was identified as a kaempferol-feruloyl-pentahexoside.

Peak 21 (t_R –22.8 min, λ_{max} – 330 nm) had a $[M-H]^-$ at *m/z* 1109, which produced on MS^2 fragments at *m/z* 947, 785 and 623 by the cleavage of respectively one, two and three hexose units. ISF analysis showed the presence of ions at *m/z* 285 and 255. This compound was therefore identified as a kaempferol-feruloyl-tetrahexoside.

Peak 22 (t_R –23.3 min, λ_{max} – 350 nm) had a $[M-H]^-$ at *m/z* 963, which yielded MS^2 fragments at *m/z* 801 ($[M-H]^-$-162, loss of a hexose), *m/z* 639 ($[M-H]^-$-324, cleavage of 2 hexose units) and *m/z* 315 ($[M-H]^-$-648, cleavage of two additional hexose moieties). On the basis of MS data, this compound was tentatively identified as a methylquercetin-tetraglucoside.

Peak 23 (t_R –23.8 min, λ_{max} – 320 nm) was a minor flavonol with a $[M-H]^-$ at *m/z* 963. MS^2 analysis showed the sequential loss of a hexose (*m/z* 755), followed by the cleavage of a 146 *amu* coumaroyl fragment (*m/z* 609) and then another hexose to produce the kaempferol aglycone at *m/z* 285. This compound was identified as a kaempferol-coumaroyl-trihexoside.

Peak 24 (t_R –26.9 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 935, consistent with a kaempferol tetrahexoside. It yielded MS^2 fragments at *m/z* 773, 447 and 285 by the successive cleavage of one, three and four hexose moieties.

Peak 25 (t_R –27.4 min, λ_{max} – 350 nm) had a $[M-H]^-$ at *m/z* 625, which with the neutral loss of a dihexoside (324 *amu*) yielded a MS fragment corresponding to quercetin at *m/z* 301. Peak 25 was therefore identified as a quercetin-dihexoside.

Peak 26 (t_R –27.8 min, λ_{max} – 520 nm) contained 2 anthocyanins. One had a $[M+H]^+$ at *m/z* 935, consistent with a cyanidin tetraglucoside. It yielded on MS^2 fragment ions at *m/z* 773, 449 and 287, corresponding to the cleavage of one, three, and four hexose units respectively. This compound was identified as cyanidin-tetrahexoside. The second anthocyanin had a $[M+H]^+$ at *m/z* 919, which produced on MS^2 fragments at *m/z* 757 ($[M+H]^+$ -162, loss of a glucosyl unit), *m/z* 449 ($[M+H]^+$-470, cleavage of two glucosyl and a coumaroyl unit) and *m/z* 287 (cyanidin, $[M+H]^+$-632, loss of an additional glucose unit). This compound was tentatively identified as cyanidin-triglucoside acylated with *p*-coumaric acid, which has been reported in red cabbage (Giusti *et al.*, 1999). This was supported by the presence of a shoulder at 320 nm in the UV spectrum.

Peak 27 (t_R –28.6 min, λ_{max} – 530 nm) was a minor anthocyanin. It had a $[M+H]^+$ at *m/z* 949, which on MS^2 produced a sequence of fragments: *m/z* 787 ($[M+H]^+$-162, loss of an hexose unit), *m/z* 449 ($[M+H]^+$-500, cleavage of 2 glucosyl and a feruloyl unit), and *m/z* 287 ($[M+H]^+$-662, loss of an additional hexose unit). This compound was tentatively identified as a cyanidin-feruloyl-triglucoside, the presence of which has been reported in red cabbage (Dyrby *et al.*, 2001).

Peak 28 (t_R –28.7 min, λ_{max} – 325 nm) was ionised in negative ion mode. It had a $[M-H]^-$ at *m/z* 965 which produced MS^2 fragments at *m/z* 803, 693, 447 and 285, characteristic of kaempferol. This compound was therefore an unknown kaempferol conjugate.

Peak 29 (t_R –30.1 min, λ_{max} – 310nm) had a $[M-H]^-$ at *m/z* 855 and MS^2 at *m/z* 693, 496 and 447. ISF analysis of this peak showed the presence of ion at *m/z* 301, which yielded an MS^2 fragment at *m/z* 255. This compound was identified as an unknown quercetin conjugate.

Peak 30 (t_R –31.3 min, λ_{max} – 520 nm) had a $[M+H]^+$ at *m/z* 919 and the same MS^2 fragments at *m/z* 757, 449 and 287 as detected in peak 26. This compound was also identified as a cyanidin-coumaroyl-triglucoside.

Peak 31 (t_R –32.0 min, λ_{max} – 520 nm) was a major peak present in purple broccoli and contained 2 different compounds. One had a $[M+H]^+$ at *m/z* 949 and an MS^2 fragmentation matched that of peak 27 with ions at *m/z* 787, 449 and 287. It was

identified as cyandin-feruloyl-triglucoside. According to the clution order it could be cyanidin-3-feruloyldiglucoside-5-glucoside, identified previously in red cabbage (Giusti *et al.*, 1999; Dyrby *et al.*, 2001). The other component had a $[M+H]^+$ at *m/z* 979, which produced on MS^2 a fragments at *m/z* 817 ($[M+H]^+$-162, loss of a glucosyl group), *m/z* 449 ($[M+H]^+$-530, corresponding to the cleavage of two glucosyl and a sinapoyl unit) and *m/z* 287 (cyanidin, $[M+H]^+$-692). On the basis of MS data and previous identification in red cabbage (Giusti *et al.*, 1999; Degenhardt *et al.*, 2000; Dyrby *et al.*, 2001), this compound was tentatively identified as cyanidin-3-sinapoyldiglucoside-5-glucoside.

Peak 32 (t_R –34.9 min, λ_{max} – 520 nm) was a minor anthocyanin, having a $[M+H]^+$ at *m/z* 1081, which yielded on MS^2 fragment ion at *m/z* 919 by the cleavage of a 162 *amu* glucosyl unit. MS^3 analysis of this ion showed the sequential cleavage of another glucosyl unit (*m/z* 757), followed by the loss of another glucosyl and a coumaroyl fragment (*m/z* 449), then another glucosyl to obtain the cyanidin aglycone (*m/z* 287). On the basis of MS data, this compound was tentatively identified as a cyanidin-coumaroyl-tetraglucoside.

Peak 33 (t_R –35.6 min, λ_{max} – 535 nm) had a $[M+H]^+$ at *m/z* 1125, which yielded on MS^2 fragment ions at *m/z* 963, 449 and 287 corresponding to the respective cleavage of a glucosyl unit, followed by a coumaroyl, a sinapoyl and another glucosyl, and finally an additional glucosyl unit. Peak 33 could be cyanidin-3-coumaroyl-sinapoyl-diglucoside-5-glucoside, the presence of which was previously reported in red cabbage (Dyrby *et al.*, 2001; Giusti *et al.*, 1999).

Peak 34 (t_R –36.3 min, λ_{max} – 535 nm) was the main anthocyanin present in red cabbage. It had a $[M+H]^+$ at *m/z* 1155, which with the loss of a 162 *amu* glucosyl fragment yielded an MS^2 at *m/z* 993. MS^3 analysis of this ion showed the sequential loss of a feruloyl unit (*m/z* 818), followed by the cleavage of both a sinapoyl and a glucosyl unit (*m/z* 449) and finally the loss of an additional glucosyl group to obtain the cyanidin aglycone at *m/z* 287. Peak 34 was identified as cyanidin-3-feruloylsinapoyl-diglucoside-5-glucoside, detected in red cabbage in earlier studies (Gisuti *et al.*, 1999; Degenhardt *et al.*, 2000; Dyrby *et al.*, 2001).

Peak 35 (t_R –37.3 min, λ_{max} – 535 nm) had a $[M+H]^+$ at *m/z* 1125, which with a loss of a 162 *amu* glucosyl fragment, yielded a MS^2 ion at *m/z* 963. MS^3 analysis of this ion yielded fragments at *m/z* 787 (993-176, loss of a feruloyl group) and *m/z* 287

(cyanidin, 993-706, cleavage of an additional glucosyl and feruloyl unit). On the basis of MS data, this compound was tentatively identified as a cyanidin-diferuloyl-triglucoside.

Peak 36 (t_R –38.7 min, λ_{max} – 330 nm) produced an $[M-H]^-$ ion at *m/z* 1477, which on MS^2 fragmented to produce a sequence of ions at *m/z* 1153 ($[M-H]^-$-324, loss of two hexose units), *m/z* 977 ($[M-H]^-$-500, cleavage of two hexose units and a feruloyl group), *m/z* 947 ($[M-H]^-$-530, cleavage of two hexose units and a sinapoyl group) and *m/z* 771 ($[M-H]^-$-706, loss of two hexose, a feruloyl and a sinapoyl group). ISF analysis of this peak showed an ion at *m/z* 977, which produced an MS^2 fragment at *m/z* 609 and 285 by the sequential loss of one and two additional hexose moieties. This compound was, therefore, identified as a kaempferol-feruloyl-sinapoyl-pentahexoside.

Peaks 37, 38, 40 and 41 (t_R –45.2 min, 46.8 min, 52.3 min and 53.9 min, λ_{max} – 325-330 nm) presented the same characteristics as peaks 21, 21, 23 and 24 in curly kale (Table 4.8). They were identified as 1,2-disinapoyl-diglucoside, 1-sinapoyl-2-feruloyl-diglucoside, 1,2,2'-trisinapoyldiglucoside and 1,2'-disinapoyl-2-feruloyldiglucoside respectively, known constituents of cauliflower and broccoli (Price *et al.*, 1997; Vallejo *et al.*, 2003; Llorach *et al.*, 2003).

Peak 39 (t_R –47.9 min, λ_{max} – 330 nm) had an $[M-H]^-$ ion at *m/z* 693, which produced MS^2 fragments at *m/z* 499 and 259, consistent with 1,2-diferuloyl-diglucoside previously identified in broccoli (Vallejo *et al.*, 2003).

Note. The presence of highly glycosylated acylated quercetin, kaempferol and cyanidin was confirmed as acid hydrolysis of purple broccoli extract produced high levels of free, monoglucoside or diglucoside quercetin and kaempferol, and cyanidin whereas peaks representing conjugates virtually disappeared (see Figure 4.21). At the same time, peaks corresponding to the modified sinapic acid and coumaroyl acid, released by acid hydrolysis appear.

4.2.2.3. Broccoli

The HPLC traces obtained at 365 and 280 nm facilitated the detection of 42 phenolic compounds (see Figure 4.12). Where reference compounds were not available identifications were facilitated by previous data on the MS fragmentation patterns of flavonols and the neutral losses associated with cleavage of various sugars and other substituent groups presented by Llorach *et al.* (2003) on cauliflower extracts (see rules

summarised in section 4.2.2.1). Identification, based on MS^2 and MS^3 data, as well as λ_{max}, are summarised below and presented in Table 4.10.

Peak 1 (t_R –10.1 min, λ_{max} – 325 nm) had a $[M-H]^-$ ion at *m/z* 353, which fragmented yielding major MS^2 fragments ion at *m/z* 191 and 179, characteristic of 3-caffeoylquinic acid (Clifford *et al.*, 2003).

Peak 2 (t_R – 14.2 min, λ_{max} – 315 nm) had a $[M-H]^-$ at *m/z* 787, which with neutral loss of three successive 162 *amu* units, yielded a MS^2 fragment corresponding to quercetin at *m/z* 301. The UV absorbance at 315 nm suggested this compound was an acylated derivative of quercetin, rather than a trihexoside conjugate. This compound was tentatively identified as quercetin-caffeoyl-dihexoside conjugate.

Peak 3 ($[t_R]$ – 14.5 min, λ_{max} – 320 nm) had a $[M-H]^-$ at *m/z* 609 which with the loss of two succesive 162 *amu* units, yielded a MS^2 fragment corresponding to kaempferol at *m/z* 285. The UV absorbance was also lower at 320 nm, suggesting that this compound was acylated. Peak 3 was tentatively identified as kaempferol-caffeoyl-hexoside on the basis of previous identification in cauliflower by Llorach *et al.* (2003).

Peak 4 and 15 (t_R –16.3 min and 23.4 min, λ_{max} – 340 nm) both had a $[M-H]^-$ at *m/z* 639 and MS^2 fragments at 315 ($[M-H]^-$-324, loss of two hexose units). Peak 15 produced an additional fragment at m/z 477, corresponding in this instance to the cleavage of a hexose unit. Compounds were tentatively identified as methylquercetin-dihexoside.

Peak 5 (t_R –16.7 min, λ_{max} – 330 nm) had a $[M-H]^-$ at *m/z* 1273, which yielded on MS^2 fragments at *m/z* 1111, 949, 787 and 625 by the sequential loss of one, two, three and four 162 *amu* units. This compound was therefore identified as a quercetin-hexahexoside, also detected in purple broccoli (peak 10, section4.2.2.2).

Peaks 6 and 16 (t_R –18.2 min and 27.4 min, λ_{max} – 340 nm) both had a $[M-H]^-$ at *m/z* 625, which on MS^2 produced a fragment ion corresponding to quercetin at *m/z* 301, The loss of 324 *amu* equates with cleavage of two glucose units. Peak 5 had an additional fragment at *m/z* 463, corresponding to the loss of a glucosyl group. Therefore peaks 6 and 16 were both quercetin-diglucosides. One may be quercetin-3-sophoroside, a known flavonol in broccoli (Price *et al.*, 1997; Plumb *et al.*, 1997).

Peak 7 (t_R –18.9 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 1257, consistent with a kaempferol-hexahexoside. The MS^2 analysis showed fragment ions at m/z 933, 771, 609

corresponding to the sequential loss of two, three and four 162 *amu* respectively. This compound was tentatively identified as a kaempferol-hexahexoside.

Peak 8 (t_R –19.4 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 1317 consistent with a quercetin pentahexoside acylated with a sinapic residue. This hypothesis was confirmed by MS fragmentation pattern. Indeed this ion produced on MS^2 fragments at *m/z* 1111 ($[M-H]^-$-206, loss of a sinapoyl), *m/z* 993 ($[M-H]^-$-324, loss of 2 hexose units), *m/z* 787 ($[M-H]^-$-530, loss of two hexose and a sinapoyl moiety).

Peak 9 (t_R –19.8 min, λ_{max} – 340 nm) had a $[M-H]^-$ at *m/z* 1155, which on MS^2 produced a sequence of fragments at *m/z* 993 ($[M-H]^-$ -162, cleavage of a hexose group), *m/z* 949 ($[M-H]^-$-206, loss of a sinapoyl unit), *m/z* 787 ($[M-H]^-$-368, loss of a sinapoyl and hexosyl units), *m/z* 625 ($[M-H]^-$-530, loss of a sinapoyl and 2 hexose groups) and *m/z* 463 ($[M-H]^-$-692, cleavage of an additional hexose group). This compound was identified as a quercetin-sinapoyl-tetrahexoside.

Peak 10 (t_R –20.2 min, λ_{max} – 345 nm) had a $[M-H]^-$ at *m/z* 1287, which on MS^2 produced ions at *m/z* 1111, 963, 787 and 625. This compound could be a quercetin-feruloyl-pentahexoside, as it had the same MS^2 fragmentation and retention time as peak 16 in purple broccoli (Table 4.9).

Peak 11 (t_R –20.6 min, λ_{max} – 345 nm) contained two compounds. One had a $[M-H]^-$ at *m/z* 1125, which on MS^2 produced fragments at m/z 963 ($[M-H]^-$-162, loss of a hexosyl unit), *m/z* 949 ($[M-H]^-$-176, loss of a feruloyl unit), *m/z* 787 ($[M-H]^-$-338, loss of both a feruloyl and a hexosyl unit) and *m/z* 625 ($[M-H]^-$-500, cleavage of a feruloyl and two hexose groups). On the basis of MS data, this compound was tentatively identified as quercetin-feruloyl-tetrahexoside. The second compound had a $[M-H]^-$ at *m/z* 1257, which produced on MS^2 a sequence of fragments: *m/z* 1111 ($[M-H]^-$-162, loss of a hexose group), *m/z* 933 ($[M-H]^-$-324, loss of 2 hexose units), *m/z* 787 ($[M-H]^-$-470 corresponding to the cleavage of 2 hexose and a coumaroyl unit) and *m/z* 625 ($[M-H]^-$-632, cleavage of an additional hexose). This compound was identified as quercetin-coumaroyl-pentahexoside.

Peak 12 (t_R –21.3 min, λ_{max} – 350 nm) produced a $[M-H]^-$ at *m/z* 1301, which produced on MS^2 fragments at *m/z* 977 ($[M-H]^-$-324 corresponding to the cleavage of two hexose groups), *m/z* 771 ($[M-H]^-$-530, corresponding to the cleavage of an additional sinapoyl unit) and *m/z* 609 ($[M-H]^-$-692, loss of an additional hexose). This compound

was identified as a kaempferol-sinapoyl-pentaglucoside, also present in purple broccoli (peak 17, Table 4.9).

Peak 13 (t_R –22.0 min, λ_{max} – 345 nm) contained two flavonols. One had a $[M-H]^-$ at *m/z* 609, which produced on MS^2 ions at *m/z* 447 and 285, corresponding in this instance to the sequential loss of one and two glucosyl units. On the basis of MS data and retention time, this compound was identified as kaempferol-3-sophoroside, a known flavonol in broccoli (Price *et al.*, 1997; Plumb *et al.*, 1997). The other component had a $[M-H]^-$ at *m/z* 1139, consistent with a kaempferol-sinapoyl-tetrahexoside previously identified in purple broccoli (peak 18, Table 4.9). MS^2 analysis showed the successive loss of two hexose moieties (*m/z* 947), and a sinapoyl unit (*m/z* 609).

Peak 14 (t_R –22.5 min, λ_{max} – 335 nm) was identified as kaempferol-feruloyl-pentahexoside, as it co-eluted with peak 20 in purple broccoli (Table 4.9), and presented the same $[M-H]^-$ at *m/z* 1271, and MS^2 fragments at *m/z* 947 and 771.

Peaks 17 and 18 (t_R –27.9 min and 31.6 min, λ_{max} – 330 and 350 nm) both had a $[M-H]^-$ at *m/z* 609, which on MS^2 produced a fragment ion corresponding to kaempferol at *m/z* 285. The 324 *amu* loss equates with the cleavage of two glucose moieties. Peak 17 produced on MS^2 an extra fragment at *m/z* 447, corresponding to the loss of a single glucose unit. Both peaks were identified as kaempferol-diglucosides.

Peak 19 (t_R –34.3 min, λ_{max} – 360 nm) is a quercetin hexose, having a $[M-H]^-$ at *m/z* 463, which with the neutral loss of a 162 *amu* yielded a MS^2 fragment corresponding to quercetin at *m/z* 301. This compound was identified as quercetin-3-glucoside, as it co-eluted with an authentic standard.

Peak 20 (t_R –34.8 min, λ_{max} – 340 nm) had a $[M-H]^-$ at *m/z* 1479, which produced on MS^2 a sequence of fragments: *m/z* 1317, 1155, 993 and 787 corresponding to the cleavage of one, two and three hexose units, followed by the loss of a sinapoyl group. On the basis of MS data, this compound was identified as quercetin-sinapoyl-hexahexoside.

Peak 21 (t_R –36.0 min, λ_{max} – 340 nm) had a $[M-H]^-$ at *m/z* 1317 consistent with a quercetin pentaglucoside acylated with a sinapic residue. This ion produced on MS^2 fragments at *m/z* 1155 ($[M-H]^-$-206, loss of a sinapoyl unit), *m/z* 993 ($[M-H]^-$-324, loss of 2 hexose units), *m/z* 787 ($[M-H]^-$-530, loss of two hexose and a sinapoyl moiety), and *m/z* 625 ($[M-H]^-$-692, corresponding to the additional cleavage of a hexose unit).

Peak 22 (t_R –36.5 min, λ_{max} – 330 nm) yielded a $[M-H]^-$ at *m/z* 1463, which on MS^2 produced fragments at *m/z* 1139, 977, 770 and 609, corresponding in this instance to

the sequential loss of two and three hexose units, followed by the cleavage of a sinapoyl and finally a further hexose group. This compound was tentatively identified as a kaempferol-sinapoyl-hexahexoside.

Peak 23 (t_R –37.0 min, λ_{max} – 340 nm) had a $[M-H]^-$ at *m/z* 1523, which on MS^2 produced ions at *m/z* 1317 ($[M-H]^-$-206, loss of a sinapoyl unit) *m/z* 1199, 993 ($[M-H]^-$-530, cleavage of a sinapoyl and two hexose units) and *m/z* 787 ($[M-H]^-$-736, corresponding to the cleavage of two hexose and a sinapoyl unit). This compound was therefore a quercetin-disinapoyl-pentahexoside.

Peak 24 (t_R –38.1 min, λ_{max} – 330 nm) had a $[M-H]^-$ at *m/z* 947, which on MS^2 produced a sequence of ions at *m/z* 785 ($[M-H]^-$-162, loss of a hexose unit), *m/z* 623 ($[M-H]^-$-324, loss of two hexose moieties), *m/z* 609 ($[M-H]^-$-338, corresponding to the cleavage of a feruloyl and a hexose group), and *m/z* 285 ($[M-H]^-$-662, cleavage of a feruloyl and three hexose units). This compound was therefore identified as kaempferol-feruloyl-trihexoside.

Peak 25 (t_R –38.5 min, λ_{max} – 330, 335 nm) contained two phenolics. One had a $[M-H]^-$ at *m/z* 1507, which yielded on MS^2 fragment ions at *m/z* 1183, 977, 771, corresponding in this instance to the successive loss of two hexose units, followed by the loss of one and then two sinapoyl groups, and a fragment at *m/z* 653 ($[M-H]^-$-854, loss of a sinapoyl and four hexose units). This compound was identified as kaempferol-disinapoyl-pentahexoside. The MS of this peak also contained an ion at *m/z* 1361 consistent with a quercetin-disinapoyl-tetraglucoside. MS^2 analysis is in agreement with this possibility as fragments ions were observed at *m/z* 1199 ($[M-H]^-$ -162, loss of an hexose unit), *m/z* 1155 ($[M-H]^-$-206 loss of a sinapoyl unit) and *m/z* 787 ($[M-H]^-$-574, loss of a hexose and two sinapoyl units).

Peak 26 (t_R – 39.5 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 1331, which on MS^2 yielded fragments at *m/z* 1169 ($[M-H]^-$-162, loss of a hexose moiety), *m/z* 1155 ($[M-H]^-$-176, loss of a feruloyl unit), *m/z* 993 ($[M-H]^-$-338, corresponding to the cleavage of a feruloyl and a hexose group), *m/z* 963 ($[M-H]^-$-368, corresponding to the cleavage of a sinapoyl and a hexose unit) and *m/z* 787 ($[M-H]^-$-544, loss of feruloyl, sinapoyl and hexose fragments). On the basis of MS data, this compound was tentatively identified as a quercetin-sinapoyl-feruloyl-tetrahexoside.

Peak 27 (t_R –39.8 min, λ_{max} – 335 nm) had a $[M-H]^-$ at *m/z* 1477, which produced on MS^2 a sequence of fragments: *m/z* 1153 ($[M-H]^-$-324, loss of two hexose units), *m/z*

977 ([M-H]$^-$-500, loss of two hexose and a feruloyl unit), *m/z* 947 ([M-H]$^-$-530, loss of two hexose units and a sinapoyl group), *m/z* 771 ([M-H]$^-$-706, corresponding to the cleavage of two hexose units, a feruloyl and a sinapoyl moicty) and *m/z* 609 ([M-H]$^-$-868, cleavage of an additional hexose unit). On the basis of MS data, this compound was identified as kaempferol-sinapoyl-feruloyl-pentahexoside.

Peak 28 (t_R –40.1 min, λ_{max} – 330 nm) showed a [M-H]$^-$ at *m/z* 1345 consistent with a kaempferol tetrahexoside acylated with two sinapic residues. The MS^2 analysis showed this ion first underwent the loss of a glucose residue (*m/z* 1183), followed by the seqential loss of two sinapoyl residues (*m/z* 977 and 771).

Peak 29 (t_R –40.6 min, λ_{max} – 330 nm) contain two components. One had a [M-H]$^-$ at *m/z* 1477, which on MS^2 produced a range of fragments at *m/z* 1153, 947 and 771, which were also observed in peak 27. This compound was therefore identified as another kaempferol-sinapoyl-feruloyl-pentahexoside. The MS of this peak also contained an ion at m/z 1331, consistent with quercetin-sinapoyl-feruloyl-tetrahexoside (see peak 26). In addition to fragments at *m/z* 1169 and 963, MS^2 analysis identified an ion at *m/z* 1125 ([M-H]$^-$-206, loss of a sinapoyl) and 949 ([M-H]$^-$-382 corresponding to the cleavage of a sinapoyl and feruloyl unit). This compound was identified as another quercetin-sinapoyl-feruloyl-tetrahexoside.

Peaks 30 and 31 (t_R –41.5 min and 42.2 min, λ_{max} – 330 nm) both had a [M-H]$^-$ at *m/z* 1315, which on MS^2 yielded fragment ions at *m/z* 1153, 977 and 771, corresponding in this instance to the sequential loss of a hexose, a feruloyl and a sinapoyl unit. Those compounds were tentatively identified as two different kaempferol-feruloyl-sinapoyl-tetrahexosides. The MS of peak 30 also contained a [M-H]$^-$ ion at *m/z* 1447, consistent with a kaempferol pentahexoside acylated with two feruloyl residues. MS^2 analysis of this ion showed the sequential loss of two hexose units (*m/z* 1123), followed by the cleavage of one and two feruloyl moieties (*m/z* 947 and 771).

Peaks 32, 33, 35 and 36 (t_R –46.3 min, 47.5 min, 54.0 min and 55.2 min, λ_{max} – 325-330 nm) presented the same characteristics as peaks 21, 22, 23 and 24 in curly kale (Table 4.8). They were tentatively identified as 1,2-disinapoyl-diglucoside, 1-sinapoyl-2-feruloyl-diglucoside, 1,2,2′-trisinapoyl-diglucoside and 1,2′-disinapoyl-2-feruloyl-diglucoside respectively, known constituents of cauliflower and broccoli (Price *et al.*, 1997; Vallejo *et al.*, 2003; Llorach *et al.*, 2003).

Peak 34 ([t_R] –48.3 min, λ_{max} – 330 nm) had a [M-H]$^-$ at *m/z* 693, which produced MS^2 fragments at *m/z* 499 and 259, consistent with 1,2-diferuloyl-diglucoside, a known constituent of broccoli (Vallejo *et al.*, 2003), previously identified in purple broccoli (see peak 39, Table 4.9).

Peak 37 (t_R –56.7 min, λ_{max} – 325 nm) had a [M-H]$^-$ at *m/z* 899, which produced MS^2 fragments at *m/z* 705, 511, 427 and 289, consistent with the presence of 1-sinapoyl-2,2-diferuloyl-diglucoside previously identified in broccoli (Vallejo *et al.*, 2003).

Note. The presence of highly glycosylated acylated quercetin and kaempferol was confirmed as acid hydrolysis of curly kale extracts produced high levels of free, monoglucoside or diglucoside quercetin and kaempferol.

4.2.2.4. Kiwi

In addition to the early eluting vitamin C peak, which was quantified in a separate ion-pair HPLC system, MS^n analysis facilitated the identification of 13 phenolic compounds in kiwi. Very little data concerning kiwi flavonoids are available in the literature, with the exception of the work of Arts *et al.* (2000) regarding the catechin content of fruits. The identification of phenolic compounds in HPLC peaks 1-16 in Figure 4.13, based on MS^2 and MS^3 data, as well as λ_{max}, are summarised below and presented in Table 4.11.

Peaks 1, 2 and 4 (t_R –8.4, 12.3 and 16.1min, λ_{max} – 290 nm) both had a [M-H]$^-$ at *m/z* 341 and a MS^2 caffeic acid fragment at *m/z* 179, produced by the neutral cleavage of 162 *amu*. These compounds were then identified as caffeic acid hexose conjugates.

Peak 3 (t_R –13.3 min, λ_{max} – 280 nm) produced a [M-H]$^-$ at *m/z* 339 which on MS^2 yielded a fragment at *m/z* 177 via a 162 *amu* cleavage. This compound was tentatively identified as a methoxycinnamic acid hexose conjugate.

Peak 5 (t_R –18.9 min, λ_{max} – 280 nm) had a [M-H]$^-$ at *m/z* 577, which produced on MS^2 fragment ions at *m/z* 425, 407 and 289 (catechin), characteristic of a catechin dimer. As this compound eluted just before (-) –epicatechin (see peak 6), it may be B2 dimer (Escribano-Bailon *et al.*, 1992; Santos-Buelga *et al.*, 1995).

Peak 6 (t_R –20.9 min, λ_{max} – 280 nm) was identified as (-)-epicatechin, as it had a [M-H]$^-$ at *m/z* 289 and MS^2 fragments at *m/z* 245, 205 and 179. This was confirmed by co-elution with a standard.

Peaks 7 and 9 (t_R · 23.0 and 33.4 min, λ_{max} – 325 and 345 nm) both had a $[M-H]^-$ at *m/z* 609, which yielded on MS^2 fragments at *m/z* 301 ($[M-H]^-$-308, loss of a rhamnosyl and glucosyl unit). Peak 7 had an extra fragment at *m/z* 463, corresponding to the loss of a rhamnosyl unit. Peak 9 was identified as quercetin-3-rutinoside, as it co-eluted with a standard, whereas peak 7 was another quercetin-hexosyl-rhamnoside, with sugars attached to the flavonol ring at differenr positions.

Peak 8 (t_R –24.0 min, λ_{max} – 280 nm) had a $[M-H]^-$ ion at *m/z* 867, which on MS^2 produced ions at *m/z* 577 by the loss of a catechin unit. It was therefore identified as a procyanidin trimer.

Peak 10 (t_R –38.4 min, λ_{max} – 345 nm) had a $[M-H]^-$ at *m/z* 593, which produced on MS^2 a fragment at *m/z* 285 (kaempferol, $[M-H]^-$-308, loss of a glucosyl and rhamnosyl unit). Peak 10 was identified as kaempferol-3-rutinoside, as it co-elutes with an authentic standard.

Peak 11 (t_R –40.0 min, λ_{max} – 340 nm) had a $[M-H]^-$ at *m/z* 447, which with the loss of a 162 *amu* glucosyl unit yielded a kaempferol ion at *m/z* 285. This compound was identified as kaemperol-3-glucoside. This identification was confirmed by co-elution with an authentic standard.

Peak 12 (t_R –40.4 min, λ_{max} · 345 nm) produced a $[M-H]^-$ at *m/z* 447 and MS^2 yielded an ion at *m/z* 301 ($[M-H]^-$-146). The MS^2 spectrum of the daughter ion at *m/z* 301 produced fragments at *m/z* 255, 179 and 151, which matched the fragmentation pattern of quercetin. On the basis of the mass spectral data this compound was identified as quercetin-rhamnoside.

Peak 13 (t_R –41.8 min, λ_{max} – 285 nm) had a $[M-H]^-$ at *m/z* 609, which on MS^2 yielded a fragment ion at *m/z* 301. This ion produced on MS^2, fragments at *m/z* 286 and 242, matching those of hesperitin. Peak 13 was identified as hesperitin-3-rutinoside (known as hesperidin). This identification was confirmed by co-chromatography with a reference compound.

4.2.2.5. Clementine

In addition to vitamin C, HPLC analysis of a clementine extract separated 12 peaks. Where standards were not available, identifications were performed using previous reports on citrus fruit flavanones and polymethoxyflavones (Robards *et al.*, 1997; Rouseff *et al.*, 1987; Dugo *et al.*, 2000) and flavonols (Kawaii *et al.*, 1999). The

identification of phenolic compounds in HPLC peaks 1-16 in Figure 4.14, based on MS^2 and MS^3 data, as well as λ_{max}, are summarised below and presented in Table 4.12.

Peak 1 (t_R –8.9 min, λ_{max} – 275 nm) had a $[M-H]^-$ at *m/z* 379, which yielded on MS^2 fragments at *m/z* 341 ($[M-H]^-$-38) and 179 (caffeic acid, $[M-H]^-$-200, loss of an additional hexose unit). This compound was identified as an unknown caffeic acid conjugate.

Peak 2 (t_R –21.4 min, λ_{max} – 325 nm) had a $[M-H]^-$ at *m/z* 401, which produced on MS^2 fragments at *m/z* 357, 277 and 233. This compound was identified as nobiletin, a known constituent of clementines (Kawaii *et al.*, 1999) and mandarins (Dugo *et al.*, 2000).

Peak 3 (t_R –22.6 min, λ_{max} – 325 nm) produced a $[M-H]^-$ at *m/z* 431 consistent with a heptamethoxyflavone, and MS^2 ions at *m/z* 401 and 270. It may be 3,3',4',5,6,7,8-heptamemethoxyflavone, as Kawaii *et al.* (1999) and Dugo *et al.* (2000) reported its presence in clementines and mandarins in previous works.

Peak 4 ($[t_R]$ –26.8 min, λ_{max} – 280 nm) had a $[M-H]^-$ at *m/z* 741, which yielded on MS^2 fragment ions at *m/z* 433 ($[M-H]^-$-308, loss of a rutinosyl) and at *m/z* 271 ($[M-H]^-$-470, loss of an additional hexose unit). On MS^3 this ion produced fragments at *m/z* 151 and 177, which matched the fragmentation pattern of naringenin. On the basis of MS data this compound was tentatively identified as a naringenin-rutinoside-hexose conjugate.

Peak 5 (t_R –31.4 min, λ_{max} – 285 nm) was a minor flavanone. It had a $[M-H]^-$ at *m/z* 595, which yielded a fragment at *m/z* 287 as the result of a 308 *amu* loss. On the basis of MS data it was identified as eridictyol-rutinoside (known as eriocitrin), present in clementine (Kawaii *et al.*, 1999).

Peak 6 (t_R –33.3 min, λ_{max} – 280 nm) had a $[M-H]^-$ at *m/z* 783, which on MS^2 produced a fragment at *m/z* 475, by cleavage of a 308 *amu* rutinosyl unit. The MS^2 of this daughter ion produced a fragment at *m/z* 271. This compound was tentatively identified as a naringenin conjugate.

Peak 7 (t_R –33.3 min, λ_{max} – 320 nm) was a quercetin rutinoside, having a $[M-H]^-$ at *m/z* 609, which with neutral loss of 308 *amu* yielded a MS^2 fragment at *m/z* 301. On MS^3 this ion produced fragments at *m/z* 271, 255 and 179, corresponding to the fragmentation pattern of quercetin. It was identified as quercetin-3-rutinoside, as it was co-eluted with a standard.

Peak 8 (t_R –37.8 min, λ_{max} – 280 nm) was a major compound in clementines. It had a $[M-H]^-$ at *m/z* 579, which on MS^2 produced an ion at *m/z* 271 ($[M-H]^-$-308, loss of a rutinosyl group). This ion produced fragments at *m/z* 151 and 177, characteristic of naringenin. Peak 8 was identified as narirutin, a known constituent of citrus fruits (Careri *et al.*, 1999; Kawaii *et al.*, 1999; Robards *et al.*, 1997). This was confirmed by co-elution with a standard.

Peak 9 (t_R –41.7 min, λ_{max} – 325 nm) was the main phenolic present in clementines. It had a $[M-H]^-$ ion at *m/z* 609, which with neutral loss of 308 *amu* yielded an MS^2 ion at *m/z* 301. The MS^3 of this ion give fragments at *m/z* 286 and 242, corresponding to the fragmentation pattern of hesperitin. This compound was identified as hesperitin-3-rutinoside, known as hesperidin. It was confirmed by co-elution with real a standard.

Peak 10 (t_R –45.2 min, λ_{max} – 325 nm) had a $[M-H]^-$ at *m/z* 763, which yielded on MS^2 fragments at *m/z* 671 ($[M-H]^-$-92) and *m/z* 301 ($[M-H]^-$-462). This ion produced on MS^3, ion fragments at *m/z* 286 and 242, characteristic of the aglycone hesperitin. On the basis of MS data, this compound was identified as a hesperitin-conjugate.

Peak 11 (t_R –54.7 min, λ_{max} – 330 nm) had a $[M-H]^-$ at *m/z* 593 and MS^2 yielded an ion at *m/z* 285 ($[M-H]^-$-308, loss of a rutinosyl group). MS^3 spectrum of the *m/z* 285 fragment produced ion at *m/z* 243 and 164, excluding the presence of kaempferol. The MS data and HPLC elution of this compound are in keeping with it being neoponcirin (isosakuranetin-3-rutinoside), the presence of which has been reported in clementine and mandarin by Robards *et al.* (1997) and Kawaii *et al.* (1999).

4.2.2.6. Green, yellow and red pepper

Peppers contained few phenolic compounds; only 9 HPLC peaks were detected for green peppers, three for yellow pepper and four for red pepper (Figure 4.15, 4.16 and 4.17). The nature of the polyphenols in the samples were very similar. Green pepper was subjected to acid hydrolysis to check the identity of the aglycone at m/z 285. Identifications were based on MS^2 and MS^3 data, as well as λ_{max}, which are summarised below and presented in Table 4.13, 4.14 and 4.15.

Peaks 1 and 2 (t_R –12.6 min and 16.4 min, λ_{max} – 285 nm) both had a $[M-H]^-$ at *m/z* 341, which yielded an MS^2 ion at *m/z* 179 (caffeic acid, $[M-H]^-$-162, cleavage of a hexose moiety). They were both identified as caffeic acid hexose conjugates.

Peak 3 (t_R - 22.8 min, λ_{max} – 345 nm) contained two compounds. One produced a $[M-H]^-$ at *m/z* 609, which on MS^2 yielded fragments at *m/z* 463 $[M-H]^-$-146, loss of a rhamnosyl unit), *m/z* 447 ($[M-H]^-$162, loss of a hexose moiety) and *m/z* 301 ($[M-H]^-$-308, loss of both a rhamnosyl and hexose fragment). This compound was tentatively identified as quercetin-rhamnosyl-hexose. The second compound, not present in green pepper, had a $[M-H]^-$ at *m/z* 771, which on MS^2 produced fragments at *m/z* 609 ($[M-H]^-$-162, loss of a hexose unit), *m/z* 463 ($[M-H]^-$-292, loss of an additional pentose unit) and *m/z* 301 ($[M-H]^-$-470, loss of two hexose and a pentose unit). This compound was identified as quercetin-pentose-dihexoside.

Peak 4 (t_R –34.7 min, λ_{max} – 330 nm) was a quercetin monoglucoside having a $[M-H]^-$ at *m/z* 463, which with neutral loss of 162 *amu* yielded MS^2 fragments at *m/z* 301 and *m/z* 179. On the basis of MS data and co-elution with a standard, this compound was identified as quercetin-3-glucoside.

Peak 5 (t_R –35.2 min, λ_{max} – 345 nm) had a $[M-H]^-$ at *m/z* 579 and MS^2 yielded ions at *m/z* 447 ($[M-H]^-$-132, loss of a pentose group) and *m/z* 285 ($[M-H]^-$-294, corresponding to the cleavage of both a hexose and pentose unit). Acid hydrolysis of green pepper released a single aglycone at m/z 285, which on MS^2 produced ions at *m/z* 241, 175 and 151, corresponding to the fragmentation pattern of lutcolin rather than kaempferol. Peak 5 was therefore identified as a luteolin-hexose-pentose conjugate.

Peak 6 (t_R –40.2 min, λ_{max} – 350 nm) was a quercetin-rhamnoside, having a $[M-H]^-$ at *m/z* 447, which produced an MS^2 ion at *m/z* 301, via the cleavage of a 146 *amu*. MS^3 of the *m/z* 301 ion produced fragments at *m/z* 271, 255, 179, which matched the fragmentation pattern of quercetin.

Peak 7 (t_R –41.6 min, λ_{max} – 280 nm) had a $[M-H]^-$ at *m/z* 609, which yielded an MS^2 fragment at *m/z* 301, which in this instance corresponds to the loss of a rutinosyl unit. MS^3 of the *m/z* 301 produced ions at *m/z* 286 and 242, characteristic of hesperitin. This compound was therefore identified as hesperidin (hespereti-3-rutinoside). This was confirmed by co-elution with a real standard.

Peak 8 (t_R –42.4 min, λ_{max} – 345 nm) had a $[M-H]^-$ at *m/z* 621, which produced on MS^2 fragments at *m/z* 489 ($[M-H]^-$-132, loss of a pentose unit) and *m/z* 285 ($[M-H]^-$-336, cleavage of an additional 204 *amu*). The loss of 204 *amu* could correspond to the loss of a hexosyl unit and a malonyl group, which on negative ionisation lost the carboxylic function (mass unit 45) before the formation of the pseudomolecular ion (Maatta *et al.*,

2003). This compound was therefore tentatively identified as luteolin-hexose-pentose-malonate.

4.2.2.7. Cherry vine tomato

In addition to vitamin C, 12 peaks were found in HPLC analyses. Where reference compounds were not available identifications were facilitated by previous data on the MS fragmentation patterns of flavonols (Stewart *et al.*, 2000; Le Gall *et al.*, 2003). The identification of phenolic compounds in HPLC peaks 1-12 in Figure 4.18, based on MS^2 and MS^3 data, as well as λ_{max}, are summarised below and presented in Table 4.16.

Peaks 1and 2 (t_R –10.8 and 15.2 min, λ_{max} – 290 nm) both had a $[M-H]^-$ at *m/z* 341, which yielded on MS^2 a caffeic acid fragment at *m/z* 179 by the cleavage of a neutral 162 *amu*. Both compounds were identified as caffeic acid hexose conjugates.

Peaks 3 and 5 (t_R –16.6 and 21.1 min, λ_{max} – 325 nm) both had a $[M-H]^-$ at *m/z* 353, which yielded on MS^2 major fragments at *m/z* 191 and 179. The ion fragment at *m/z* 179 was much stronger in peak 3 compared to peak 5. On the basis of MS data and according to the criteria of Clifford *et al.* (2003), peak 3 was identified as 3-caffeoylquinic acid and peak 5 as 5-caffeoylquinic acid.

Peak 4 (t_R –19.4 min, λ_{max} – 340 nm) produced a $[M-H]^-$ at *m/z* 771, which yielded on MS^2 fragment ions at *m/z* 609 ($[M-H]^-$-162, loss of an hexose) and *m/z* 301 (quercetin, $[M-H]^-$-470, loss of a hexose and a rutinose group). This compound was tentatively identify as a quercetin-rutinoside-glucoside, possibly quercetin-3-rutinoside-7-glucoside, as Le Gall *et al.* (2003) have reported the presence of kaempferol-3-*O*-rutinoside-7-*O*-glucoside in genetically modified tomato fruit.

Peak 6 (t_R –30.7 min, λ_{max} – 355 nm) produced a $[M-H]^-$ at *m/z* 741, which on MS^2 produced fragments at *m/z* 609 and 301. These ions were formed by the successive loss of a pentose and a rutinose unit. Peak 6 was identified as quercetin-rutinoside-pentoside.

Peak 7 (t_R –33.3 min, λ_{max} – 355 nm) was the main polyphenol present in cherry tomato. It was identified as quercetin-3-rutinoside (rutin), a known constituent of tomatoes (Stewart *et al.*, 2000). It had a $[M-H]^-$ at *m/z* 609 producing a MS^2 ion at *m/z* 301 by the cleavage of a 308 *amu*. This was confirmed by co-elution with a standard.

Peak 8 (t_R –34.6 min, λ_{max} – 325 nm) produced a $[M-H]^-$ ion at *m/z* 725, which on MS^2 yielded fragments at *m/z* 593 ($[M-H]^-$ -132, loss of a pentose unit) and *m/z* 285 ($[M-H]^-$ -440, corresponding to the successive cleavage of a pentose and a rutinose group). This compound was tentatively identified as kaempferol-rutinoside-pentose.

Peak 9 (t_R –38.4 min, λ_{max} – 330 nm) had a $[M-H]^-$ ion at *m/z* 593 and a MS^2 kaempferol fragment ion at *m/z* 285, produced by cleavage of a 308 *amu* rutinosyl unit. Peak 9 was identified as kaempferol-3-rutinoside, as it co-eluted with a standard.

Peak 10 (t_R –43.2 min, λ_{max} – 325 nm) had a $[M-H]^-$ ion at *m/z* 515 and produced on MS^2 a main fragment at *m/z* 353 and a minor one at *m/z* 173. The MS data are consistent with the presence of 3,4-dicaffeoylquinic acid (Clifford *et al.*, 2003).

Peak 11 (t_R –57.5 min, λ_{max} – 330 nm) had a $[M-H]^-$ at *m/z* 677, which on MS^2 yielded a fragment ion at *m/z* 515 (loss of a 162 *amu*) consistent with a caffeoyl unit. On MS^3, this ion produced fragment at *m/z* 353, 335, 179 and 173, which corresponds the fragmentation pattern of a dicaffeoylquinic acid (Clifford *et al.*, 2003). This compound was tentatively identified as a tricaffeoylquinic acid. Presence of a tricaffeoylquinic acid has been previously detected in sweet potato (Yoshimoto *et al.*, 2002).

Peak 12 (t_R –61.9 min, λ_{max} – 280 nm) had a $[M-H]^-$ at *m/z* 271 and MS^2 fragments at *m/z* 151 and 177, which were consistent with naringenin. Co-elution with an authentic standard and previous work (Le Gall *et al.*, 2003) confirmed this identification.

4.2.2.8. Plums

In plums, 30 peaks were found in HPLC traces, corresponding to 32 individual phenolic compounds (Figure 4.19). Most were polymeric flavan-3-ols, which were not specifically characterised in this study. Indeed procyanidins polymers do not chromatograph well as seen in Figure 4.19, causing a bump in the 280 nm trace. This can cause problems with MS analysis as multiply charged ions from the polymers can interfere with single charged ion from smaller dimer and trimer peaks. For the other phenolics, where standards were not available, identifications were assisted by previous reports on plums (Tomas-Barberan *et al.*, 2001; Chun *et al.*, 2003). The identification of phenolic compounds in peaks 1-30, based on MS^2 and MS^3 data, as well as λ_{max}, are summarised below and presented in Table 4.17.

Peaks 1, 7, 9, 14 and 24 (t_R –13.0 min, 17.7 min, 19.1 min, 24.1 min and 33.7 min, λ_{max} – 280 nm) had a $[M-H]^-$ at *m/z* 577, which produced MS^2 fragment ions at *m/z*

425, and 289 (catechin or epicatechin), characteristic of catechin dimers. Tomas-Barberan *et al.* (2001) reported the presence of dimers B1, B4 and B7 and other unidentified catechin dimers in plums.

Peak 2 (t_R –13.9 min, λ_{max} – 280 nm) had a $[M-H]^-$ at *m/z* 1439 and produced on MS^2 a sequence of fragments: *m/z* 1151 ($[M-H]^-$ 288, loss of a catechin monomer), *m/z* 863 ($[M-H]^-$-576, loss of two catechin/epicatechin monomers), *m/z* 575 ($[M-H]^-$-864, loss of three catechin/epicatechin monomers) and minor fragments were seen at *m/z* 1989 and *m/z* 1672. The presence of those minor fragments suggested this ion may have been multicharged. Peak 2 was therefore a polymer of catechin of at least five units.

Peaks 3 and 11 (t_R –15.2 min and 21.2 min, λ_{max} – 280 nm) both had a $[M-H]^-$ at *m/z* 289, which produced MS^2 fragments at *m/z* 245 and 179. Peak 3 was identified as (+)-catechin and peak 11 as (-)-epicatechin and this was confirmed by co-elution with authentic standards.

Peak 4 (t_R –15.8 min, λ_{max} – 280 nm) had a pseudomolecular ion at *m/z* 575, which yielded on MS^2 analyses a fragment ion at *m/z* 287 by cleavage of a 288 catechin group. This compound was identified as a A-type dimer of catechin (loss of an additional -H group), the presence of which has been previously reported in plums by Tomas-Barberan *et al.* (2001).

Peaks 5 and 23 (t_R –16.4 min and 33.3, λ_{max} – 280 nm) both had a $[M-H]^-$ ion at *m/z* 1441, consistent with a pentamer of catechin, which on MS^2 yielded fragments at *m/z* 1153, 865 and 577, by the sequential cleavage of one, two and three monomeric catechin units. Both compounds were, therefore, identified as B-type pentamers of catechin.

Peak 6 (t_R –16.9 min, λ_{max} – 280 and 515 nm) contained two phenolic compounds. One had a $[M+H]^+$ at *m/z* 449, which on MS^2 produced a cyanidin fragment at *m/z* 287 ($[M+H]^+$-162, cleavage of an hexose unit). This compound was identified as cyanidin-3-glucoside, a known constituent of plums (Chun *et al.*, 2003; Tomas-Barberan *et al.*, 2001) and confirmed by co-elution with an authentic standard. Negative ionisation of peak 6 revealed the presence of a $[M-H]^-$ at *m/z* 865, which yielded MS^2 fragments at *m/z* 577 ($[M-H]^-$-288, loss of a catechin unit) and *m/z* 695. It was identified as a trimer of catechin.

Peak 8 (t_R –18.4 min, λ_{max} – 280 and 515 nm) also contained two compounds. One had a $[M+H]^+$ ion at *m/z* 595, which after the sequential cleavage of a rhamnosyl and a glucosyl moiety, yielded a cyanidin MS^2 fragment at *m/z* 287. This compound was

therefore cyanidin-3-rutinoside, the main anthocyanin in plums (Chun *et al.*, 2003; Tomas-Barberan *et al.*, 2001). The second compound had a $[M-H]^-$ ion at *m/z* 865, which produced fragments at *m/z* 577 ($[M-H]^-$-288, loss of a catechin unit). However ions at *m/z* 695 and 1309 suggest this compound was multicharged. It was identified as a polymer of catechin.

Peaks 10, 19 and 21 (t_R –20.4 min, 27.4 min and 29.8 min, λ_{max} – 280 and 515 nm) had identical mass spectra with a $[M-H]^-$ at *m/z* 1729, consistent with a hexamer of catechin. This possibility was confirmed by the MS fragmentation pattern, as it yielded MS^2 fragment ions at *m/z* 1441, *m/z* 1271, *m/z* 1153 and *m/z* 575 by the successive loss of one, two, three and four monomers of catechin units.

Peak 12 (t_R –22.2 min, λ_{max} – 280) had a multicharged ion at *m/z* 865, which produced MS^2 fragment ions at *m/z* 575 ($[M-H]^-$-288, cleavage of a flavan-3-ol monomer) and *m/z* 1605, *m/z* 1441 and *m/z* 1152. It was also identified as a polymeric proanthocyanidin.

Peaks 13, 15 and 17(t_R –23.2 min, 24.7 min, and 25.9 min, λ_{max} – 280) all had a $[M-H]^-$ at *m/z* 1441, which produced on MS^2 ion at *m/z* 1151 ($[M-H]^-$-288, loss of a catechin unit), *m/z* 865 ($[M-H]^-$-576, cleavage of an additional catechin), *m/z* 576 ($[M-H]^-$ -864, cleavage of three catechin units), as well as higher mass fragments (*m/z* 1847 and 1710 like peak 13, *m/z* 1891 like peak 15 and *m/z* 1889 like peak 17). Those ions were therefore multicharged and compounds were identified as polymeric proanthocyanidins.

Peak 16 (t_R –25.2 min, λ_{max} – 280) had a $[M-H]^-$ ion at *m/z* 1729, which was multicharged as it produced large fragments at *m/z* 1603 and 1891. By the successive cleavage of one, two and three catechin units, this ion produced MS^2 fragments at *m/z* 1440, 1151 and 863, characteristic of a proanthocyanidin. Peak 16 was therefore a polymeric catechin.

Peak 18 (t_R –26.7 min, λ_{max} – 280) had a multicharged pseudomolecular ion at *m/z* 867 and on MS^2 produced a fragment at *m/z* 1114 and a sequence of ions at *m/z* 741, 579 ($[M-H]^-$-288, loss of a catechin unit), *m/z* 497, *m/z* 287 ($[M-H]^-$-580, loss of two catechin units). It was also a polymeric proanthocyanidin.

Peak 20 (t_R –28.6 min, λ_{max} – 280) was a minor flavan-3-ol, having a $[M-H]^-$ ion at *m/z* 865. It produced MS^2 ions at *m/z* 739, *m/z* 695, *m/z* 577 ($[M-H]^-$ -288, loss of a catechin unit), *m/z* 289 ($[M-H]^-$ -576, loss of 2 catechin groups), *m/z* 1151 and *m/z* 1439. This compound was also multicharged and was a polymer of catechin.

Peak 22 (t_R –32.9 min, λ_{max} – 355) had a $[M-H]^-$ ion at *m/z* 609, which with a 308 *amu* loss, produced on MS^2 fragment at *m/z* 301. MS^3 analysis of this ion yielded fragments at *m/z* 271, 255 and 179, characteristic of the aglycone quercetin. Peak 22 was identified as quercetin-3-rutinoside, previously found in plums (Tomas-Barberan *et al.*, 2001). This was confirmed by co-elution with a standard.

Peak 25 (t_R –34.4 min, λ_{max} – 355) was a quercetin-monoglucoside, having a $[M-H]^-$ at *m/z* 463, which on MS^2 produced an ion at *m/z* 301. MS^3 of the *m/z* 301 fragment produced ions at *m/z* 271, 255 and 179. On basis of the MS data, co-elution with a standard, peak 25 was identified as quercetin-3-glucoside.

Peaks 26 and 28 (t_R –36.6 min and 39.8 min, λ_{max} – 355) had identical mass spectra with a $[M-H]^-$ at *m/z* 433, which yielded an MS^2 fragment at *m/z* 301, which in this instance corresponded to the loss of a pentose unit ($[M-H]^-$-132). These compounds were therefore identified as quercetin-pentosides.

Peak 27 (t_R –36.9 min, λ_{max} – 350) had a $[M-H]^-$ ion at *m/z* 565, which produced MS^2 fragment ions at *m/z* 415 and 301 ($[M-H]^-$-264, loss of two pentose units). This compound was identified as quercetin pentosyl-pentoside, the presence of which was previously reported in plums (Tomas-Barberan *et al.*, 2001).

Peak 29 (t_R –40.2 min, λ_{max} – 350) had a $[M-H]^-$ ion at *m/z* 447, with MS^2 producing a fragment at *m/z* 301 ($[M-H]^-$-146, loss of a rhamnosyl moiety). On the basis of the MS data and previous work (Tomas-Barberan *et al.*, 2001), this compound was tentatively identified as quercetin-3-rhamnoside.

Peak 30 (t_R –41.6 min, λ_{max} – 355) produced a $[M-H]^-$ ion at *m/z* 505, which with loss of 204 *amu* yielded a quercetin MS^2 fragment at *m/z* 301. This corresponded to the loss of a hexosyl unit and a malonyl group, which in negative ion mode lost the carboxylic function (mass unit 45) from the pseudomolecular ion (Maatta *et al.*, 2003). MS^3 analysis of the ion at *m/z* 301 showed fragments at *m/z* 255, 179 and 151, confirming the presence of quercetin. This compound was tentatively identified as quercetin-malonate-hexoside.

4.2.3. Identification of carotenoids using HPLC analysis

Carotenoids and tocopherol were identified and quantified by comparison with commercial standards. The antioxidant potential of fruits and vegetables (except broccoli

and purple broccoli, which have not been analysed) was carried out using the HPLC-DPPH method. Curly kale, green, yellow and red pepper contained significant levels of carotenoids and tocopherol. Figures 4.23 to 4.25 show profiles of vegetables analysed for carotenoids and tocopherol. All the carotenoids are expressed as β-carotene equivalents and tocopherols as α-tocopherol equivalents (see Tables 4.25, 4.30, 4.31 and 4.32).

4.2.4. Quantification of fruit and vegetable antioxidants and their respective antioxidant capacity

Quantitative estimates of all antioxidants are presented in Tables 4.25 to 4.34. Antioxidant results are expressed in Trolox equivalents. For the quantification of phenolic compounds, all phenolic acids (hydroxycinnamates and hydroxybenzoic conjugates) were expressed as 4-hydroxybenzoic acid equivalents, all flavan-3-ols and their polymer in (+)-catechin equivalents, anthocyanin conjugates in cyanidin-3-glucoside equivalents, quercetin conjugates in quercetin-3-glucoside equivalents, kaempferol conjugates in kaempferol-3-glucoside equivalents, myricetin conjugate in myricetin equivalents, ellagic acid conjugates in ellagic acid equivalents, flavanones in hesperitin equivalents and flavones in apigenin equivalents. For each compound the percentage contribution to the total antioxidant activity (% AOX) was calculated and reported in the Tables.

4.2.5. Discussion

Curly kale

Curly kale contained highly glycosylated acylated flavonoids, mainly quercetin and kaempferol derivatives, as was previously detected in cabbage leaves (Nielsen *et al.*, 1998) and in cauliflower (Llorach *et al.*, 2003). In the present study, flavonols were the main flavonoids present with a total of 604 μg/g quercetin conjugates and 593 μg/g kaempferol conjugates. These values are much higher than the 77-244 μg/g and 235-347 μg/g of quercetin and kaempferol aglycones respectivly reported by Zhang *et al.* (2003) after acid hydrolysis. After similar acid hydrolysis conditions, we were still able to detect quercetin and kaempferol- glucoside and diglucoside (*ca* 60 μg/g and 288 μg/g of quercetin diglucoside and monoglucoside, and 294 μg/g and 56 μg/g of kaempferol diglucoside and monoglucoside) in addition to the quercetin and kaempferol aglycones

(79 μg/g and 200 μg/g respectively). Some sinapic acid ester conjugates were also present (44.6 μg/g in total); similar compounds have been previously reported in broccoli or cauliflower (Vallejo *et al.*, 2003; Llorach *et al.*, 2003). We also indentified some 3-caffeoylquinic acid (but this was not quantified as it coeluted with a quercetin conjugate) as reported by Sakakibara *et al.* (2003). These authors also reported the presence of 12 nmol/g luteolin glycosides in cabbage, which were not detected in the present study, as kaempferol was the sole aglycone found having a pseudomolecular ion at m/z 285. This was in agreement with Justesen *et al.* (1998). Flavonols were the main contributors to the antioxidant activity of curly kale (38.5%); in particular the quercetin conjugates contributed 29.9% of antioxidant capacity. Sinapic acid conjugates also contributed 19.9% of the total antioxidant activity.

Broccoli

Analysis of purple broccoli and broccoli revealed the presence of the same kind of highly glycosylated and acylated flavonols with a total content of 370 μg/g of quercetin and 469 μg/g of kaempferol conjugates for purple broccoli and 158 μg/g and 267 μg/g of quercetin and kaempferol conjugates respectively for broccoli. The nature of those compounds was quite different compared to curly kale or cauliflower. After acid hydrolysis, we identified *ca* 340 μg/g quercetin and 405 μg/g kaempferol in purple broccoli, 110 and 214 μg/g of quercetin and kaempferol in broccoli. These results confirm our identifications: kaempferol and quercetin are present as a range of highly glycosyated and acylated conjugates in purple broccoli and broccoli. Price *et al.* (1998) reported the most abundant flavonol glycosides in broccoli were the sophorosides of quercetin (65 μg/g) and kaempferol (166 μg/g). In the present study the diglucosides of quercetin and kaempferol were present in broccoli, but in much lower amounts (with 23 μg/g and 51 μg/g respectively) and flavonol sophorosides represented only 17.4 % of the total flavonol content of broccoli. Broccoli and to a lesser extent purple broccoli was a good source of cinnamic acid derivatives (with 223 μg/g and 71 μg/g respectively), mainly present as 1-sinapoyl-2-feruloyldiglucoside, 1,2′-disinapoyl-2-feruloyl diglucoside and 1,2′-disinapoyl diglucoside. The presence of such compounds in broccoli has been reported in previous reports (Plumb *et al.*, 1997; Vallejo *et al.* 2003). Purple broccoli also contained anthocyanins, which are likely responsible for the reddish colour. Interestingly the profile of anthocyanins is very complex, showing different acylated and glycosylated patterns. Acid hydrolysis of the extract confirmed that cyanidin was the only

aglycone present. The presence of similar anthocyanins has been reported in red cabbage (Degenhardt *et al.*, 2000; Dyrby *et al.*, 2001 and Stintzing *et al.*, 2002). Their common basic structural feature was a cyanidin-3-sophoroside-5-glucoside or a cyanidin-3,5-diglucoside backbone. In the present study, purple broccoli contained in total 357 μg/g of anthocyanin, the main compound being cyanidin-3-feruloyl-sinapoyl-diglucoside-5-glucoside (107 μg/g). Broccoli also contains glucosinolates (Tolra *et al.*, 2000), which were not investigated in the present study.

Flavonols were the main flavonoids present in both broccoli and purple broccoli and they contributed to 32.9 % of the total antioxidant activity in broccoli but only to 12.7 % in the purple variety, where anthocyanins were the main contributors (24.7 %), followed by cinnamic acid derivatives (14.4 %). In traditional green broccoli, hydroxycinnamates were the main contributors to antioxidant activity (44.0 %), followed by flavonols. Interestingly, when TEAC (Trolox equivalent antioxidant capacity, defined as the mM concentration of Trolox having equivalent activity to a 1mM concentration of the compound investigated) of acylated anthocyanins was calculated, values appear to be very high (1.0 for cyanidin-coumaroyl-tetraglucoside up to 7.2 for cyanidin-sinapoyl-feruloyl-triglucoside. Previous work with berries has shown that anthocyanins, attached to sugar moieties had TEAC around 0.8-1.2 (section4.1). This is in accordance with Stintzing *et al.* (2002), who reported that acylations of anthocyanins with cinnamic acids markedly increased their antioxidant activity.

Kiwi

Kiwi contained very low levels of phenolics including 45.2 μg/g of hydroxycinnamate conjugates, 28 μg/g of epicatechin and condensed catechin (dimer or trimer of catechin/epicatechin) and 3.8 μg/g flavonols (kaempferol and quercetin conjugates). The main antioxidant was vitamin C, which contributed to 68.7% of the overall antioxidant activity with a content of 585 μg/g, whereas other polyphenols, principally flavan-3-ols, contributed only 5.8% (5.2% due to flavanols).

Clementines

The major polyphenols in clementines were flavanones, mainly present as hesperidin (299 μg/g) and narirutin (92 μg/g). Similar levels of hesperitin (366 μg/g) and naringenin (106 μg/g) were reported by Roberts *et al.* (2003). We additionally found some narirutin glucoside, eriocitrin and neoponcirin, in agreement with Robards *et al.* (1997) and

Kawaii *et al.* (1999), and low levels of unknown conjugates of both hesperitin and naringenin. Interestingly, these compounds were very poor antioxidants and contributed only 1.6 % of the total antioxidant activity. The flavones nobiletin and heptamethoxyflavone, were the only flavonoids to contribute significantly to the total antioxidant activity (9.2 %). In addition to flavones, Dugo *et al.* (2000) reported the presence of sinsensetin and tetra-*O*-methylscutellarein in clementines, which were not detected in the present study. Vitamin C (323 μg/g) was the main contributor to antioxidant activity with 61.5 %. Roberts *et al.* (2003) found some β-cryptoxanthin and β-carotene in clementines but thes carotenoids were not detected in our methanolic extracts.

Peppers

Green peppers contained higher levels of polyphenolics as compared to the other varieties, principally as flavonols (30 μg/g of quercetin conjugates), and flavones (38 μg/g luteolin conjugates), whereas red and yellow peppers contained 0.9 and 0.3 μg/g quercetin conjugates respectively and 7.9 and 10.9 μg/g of luteolin conjugates. Green pepper also contained some hydroxycinnamates (5.1 μg/g). All of the peppers contained low levels of hesperidin (3.8 μg/g in yellow pepper up to 10.1 μg/g in green variety). Similarly Justesen *et al.* (1998) reported that green peppers (but not red or yellow pepper) contained quercetin conjugates. Vitamin C was the main contributor to antioxidant activity (88 % in both green and yellow pepper, 69.8 % in red peppers), whereas flavonoids had little impact on antioxidant capacity, contributing 0.6 %, 1.1 % and 3.9 % for red, yellow and green pepper respectively, due mainly to the presence of luteolin conjugates. Carotenoids and tocopherols were present mainly in red pepper where they contributed 22.3 % of the total antioxidant activity. Most of the carotenoids mentioned in the present study (tentatively identified on the basis of their UV absorbance (between 450-470 nm) and later elution in an organic phase) were not identified, as we did not have access to appropriate standards, and MS characterization was not possible. Sixteen carotenoids were present in red pepper, including β-cryptoxanthin (23 μg/g), as well as 194 μg/g of α-tocopherol. Peak 4 could be capxanthin. Saltmarsh *et al.* (2003) reported capsanthin is the bright red pigment present in red pepper, whereas, lutein is the colourant of yellow peper. Roberts *et al.* (2003) reported a far higher level in α-tocopherol in peppers (14170 μg/g) than observed in the present study (194 μg/g). They also detect the presence of lutein, α and β-carotene, which were not identified in the present study.

However, our method of extraction (in methanol) was not particularly appropriate for carotenoids, which are lipophilic.

Cherry tomato

In cherry tomatoes, vitamin C was the major antioxidant (58 %). Nonetheless phenolic compounds contributed to 19 % of the antioxidant activity. Indeed mono-, di- and tri-caffeoylquinic acids (32 μg/g in total) were strong antioxidants, contributing to 10 % of the total antioxidant activity. Flavonols (79 μg/g in total), mainly present as rutin (66 μg/g) were in comparison less effective antioxidants, but as they were present in higher levels, represented 9.1% of the total antioxidant activity. These values are in agreement with previous publications. After acid hydrolysis, Martinez-Valverde *et al.* (2002) reported the presence of 14.3 - 32.8 μg/g of chlorogenic acid, 7.2 - 43.6 μg/g quercetin; Crozier *et al.* (1997) reported the presence of 2 - 203 μg/g quercetin, with higher levels in cherry tomatoes.

No carotenoids were found in the methanolic extract, probably due to the method of extraction. Martinez-Valverde *et al.* (2002), who solubilised the carotenoids in a mix of hexane/acetone/ethanol, reported 18.6 to 64.9 mg/kg of lycopene in different varieties of tomato.

Plum

In plums, high levels of flavan-3-ols were found (1202 μg/g catechin equivalent), mainly present as polymers of catechin. Tomas-Barberan *et al.* (2001) reported the presence of procyanidins (dimers B1, B4 and B2, as well as two A-type dimers, and other unidentified dimers or trimers) in plum cultivars. The content reported was 662 - 1837 μg/g in the peel and 156 - 618 μg/g in the flesh. Plums were also a rich source of anthocyanins present as cyanidin-3-glucoside (53 μg/g) and cyanidin-3-rutinoside (20 μg/g). They also contained some quercetin conjugates (123 μg/g). These values were in accordance with previous work: Chun *et al.* (2003) reported the presence of 127 - 703 μg/g of cyanidin conjugates and 10 to 128 μg/g of quercetin conjugates in different plum cultivars (11 in total). Several investigations have reported the presence of chlorogenic acids in plums (Roberts *et al.*, 2003; Tomas-Barberan, 2001; Chun *et al.*, 2003), which were not found in our extracts. Roberts et al. (2003) also reported the presence of 360 mg/100g α-tocopherol and 101 mg/100g β-carotene, which we were not able to identify

in the present study. Flavan-3-ols are the main contributors to antioxidant activity (70 %), followed by anthocyanins (contributing less than 11 %) and flavonols (5.3 %).

Using a combination of techniques. HPLC, MS and on-line measurement of antioxidant activity, we were able to identify a wide range of bioactive compounds in the 10 fruits and vegetable analysed. Nonetheless, our results were based on the analysis of a single variety and are not representative of the whole species. Indeed the content of phenolics and vitamins in fruits and vegetables is affected by numerous factors such as variety, soil, climatic conditions, degree of ripeness or storage conditions (Crozier, 2003). Furthermore, the method of extraction and the choice of solvents also has an impact on the results (Heinonen *et al.*, 1998). For example, the level of carotenoids reported in the present study is more likely underestimated, as those compounds are lipophilic and are not fully extracted by methanol.

Some forms of cooking are also recognised to affect the concentrations of the bioactive compounds. This certainly applies to broccoli and kale which require cooking before consumption). Indeed Crozier *et al.* (1997) reported that the quercetin in onions and tomatoes was affected to varying degrees by cooking: boiling reduced the content by 80%, microwave cooking by 65% and frying by 30%. Similarly, Price *et al.* (1997) reported that only 14-28% of the flavonols present were retained in boiled broccoli. This has to be taken in account when estimating dietary intakes.

4.3. Conclusions

To date most of studies dealing with the identification of polyphenolic compounds present in fruits and vegetables were indirect determinations of aglycones after acid hydrolysis (Hertog *et al.*, 1992; Justesen *et al.*, 1998; Hakkinen *et al.*, 1999), or focused on a particular class of phenolic compounds (Goiffon *et al.*, 1991 and 1999; Degenhardt *et al.*, 2000 for anthocyanins; Schuster et al., 1985 for phenolic acids; Arts *et al.*, 2000 for catechin). More recently, Sakakibara *et al.* (2003) were simultaneously analysed "all" the polyphenols (100 standards) in 63 samples (fruits, vegetables and tea samples) using an HPLC method. Our work takes this identification process a step further: by using a system combining HPLC, MS^n and on-line assessment of antioxidant activity, we could identifiy the bioactive compounds (vitamin C, carotenoids, poylphenols) present in a range of 17 fruits and vegetables and determine their direct contribution to the total

antioxidant activity. In the present study, the fruits and vegetables analysed were a rich source of antioxidants, which along with the minerals present, may be the basis for the beneficial effect of these foods. However, the occurrence of phenolic compounds, carotenoids and vitamin C and their direct contribution to the total antioxidant potential varies significantly between different produce analysed. If the epidemiological evidence for the benefit of consuming diets rich in fruits and vegetables is quite compelling, evidence for specific fruits or vegetables, or specific compounds is less convincing.

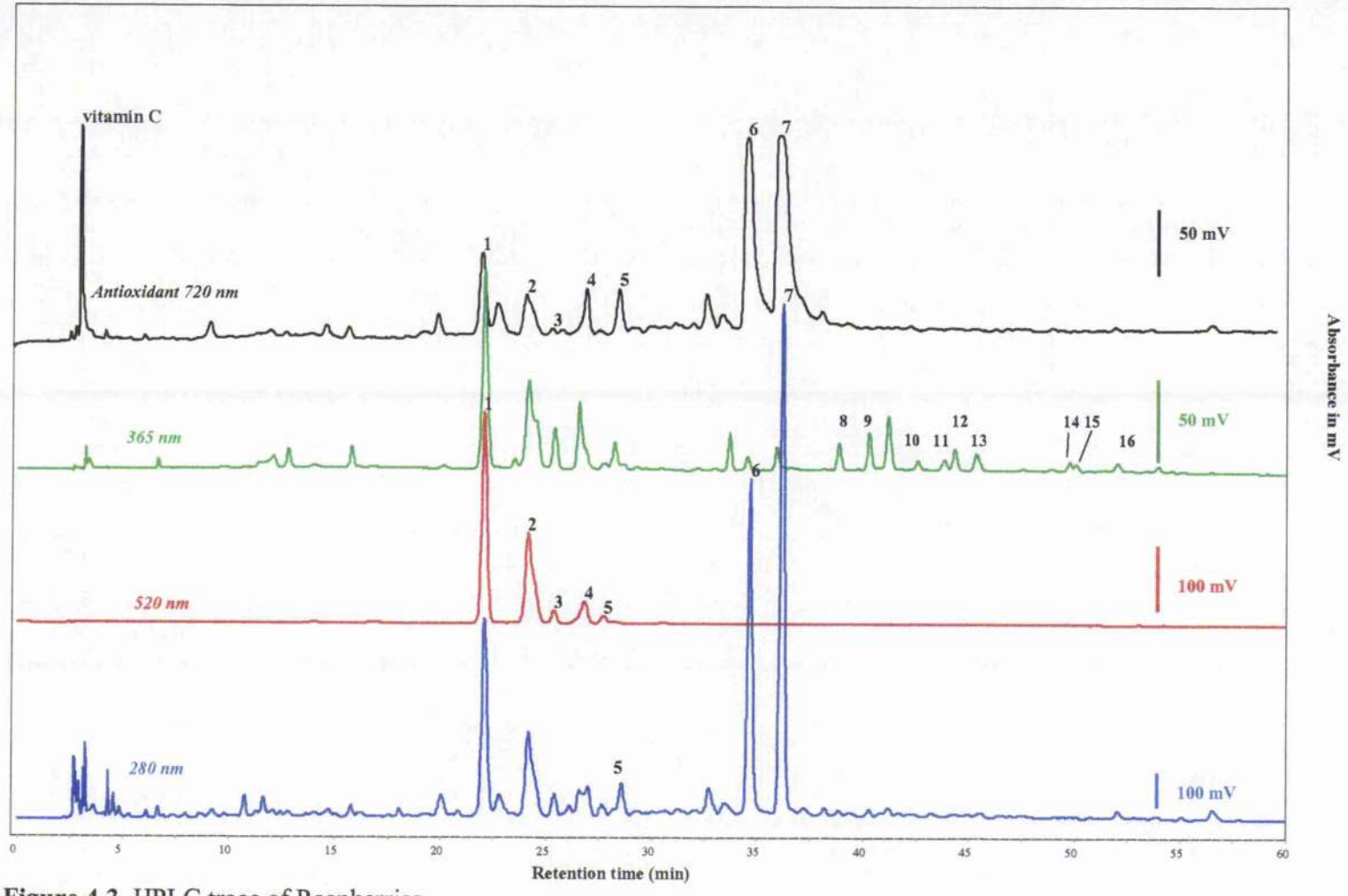


Figure 4.3. HPLC trace of Raspberries

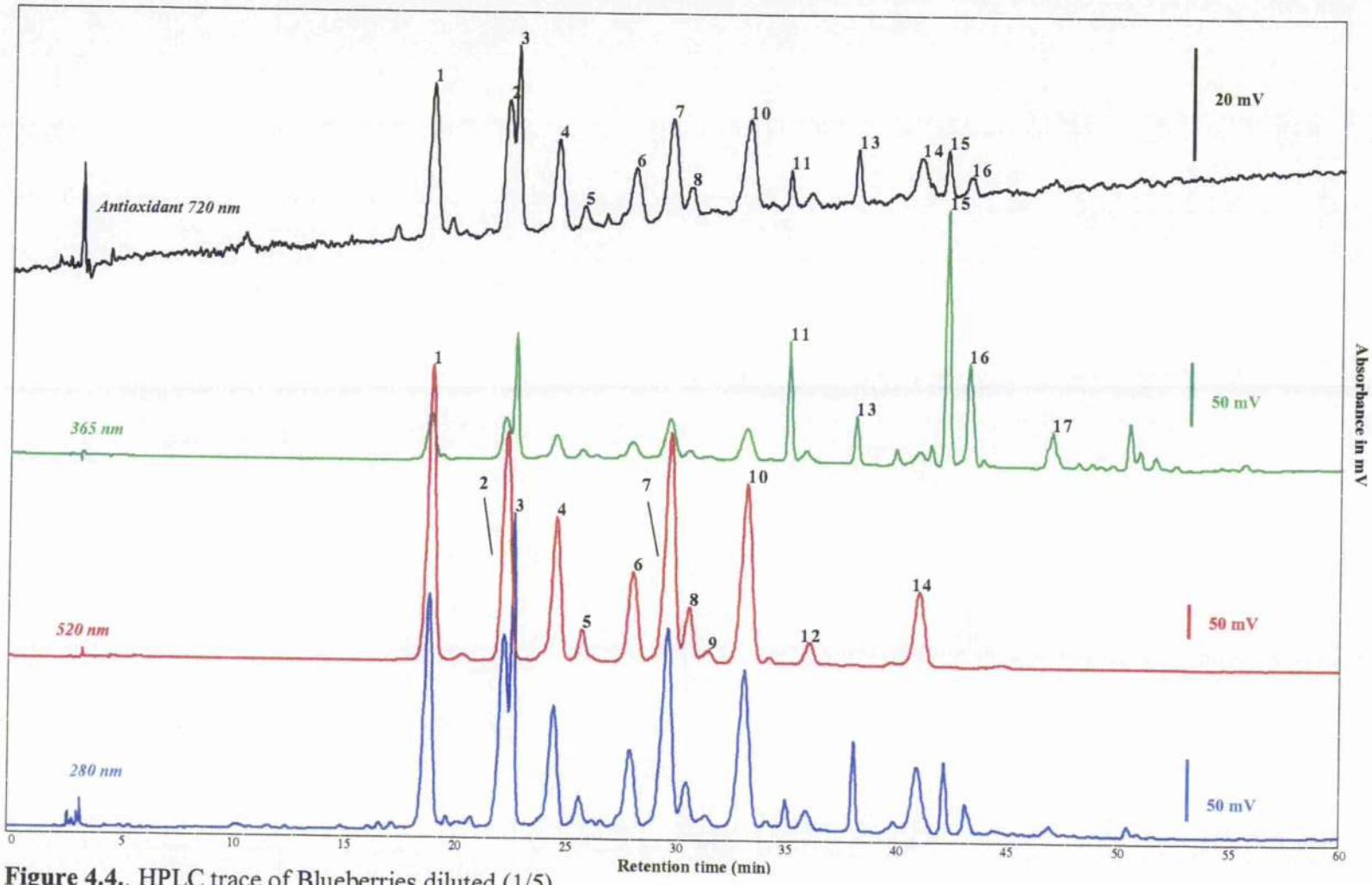


Figure 4.4.. HPLC trace of Blueberries diluted (1/5)

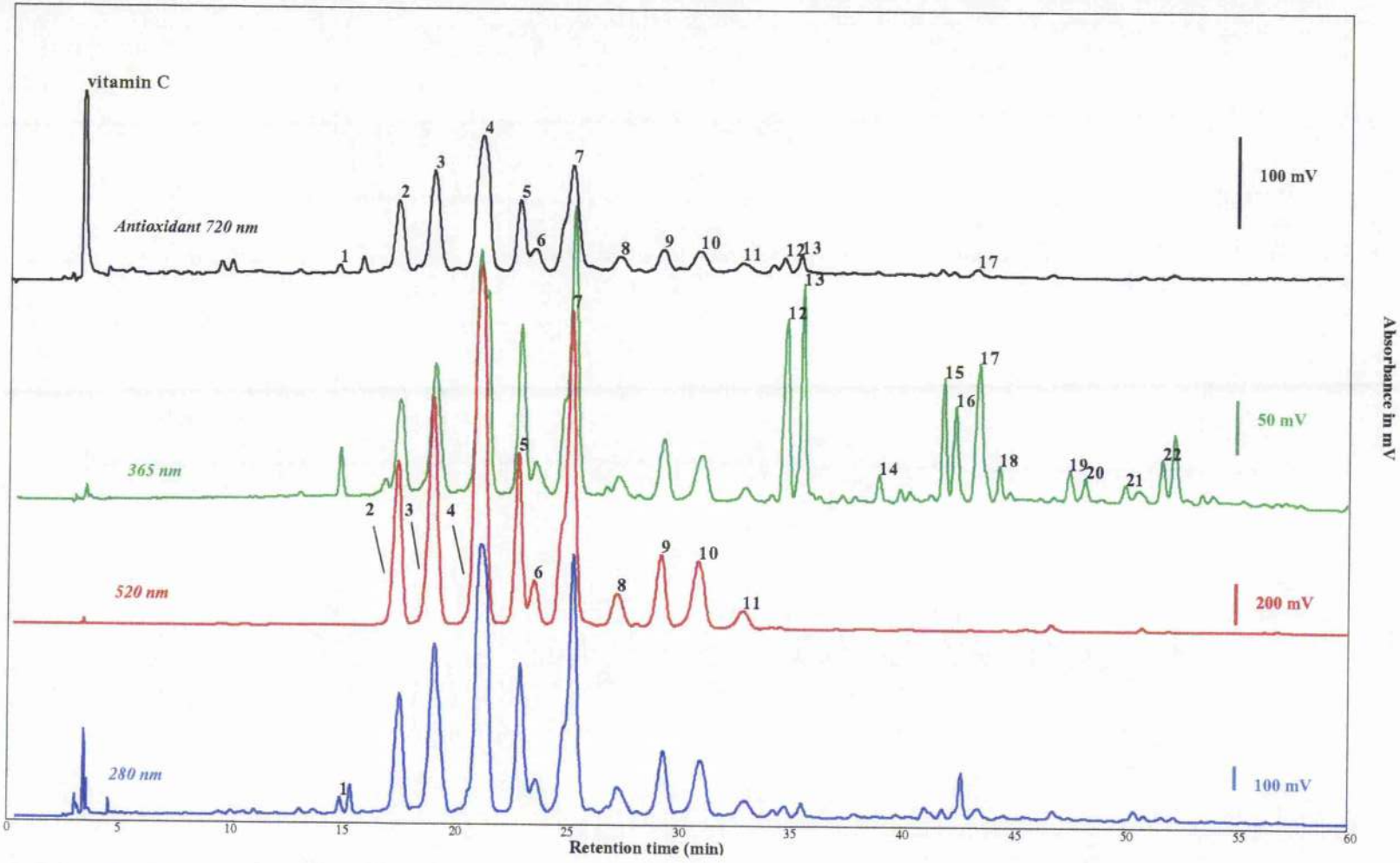


Figure 4.5. HPLC trace of Blackcurrants

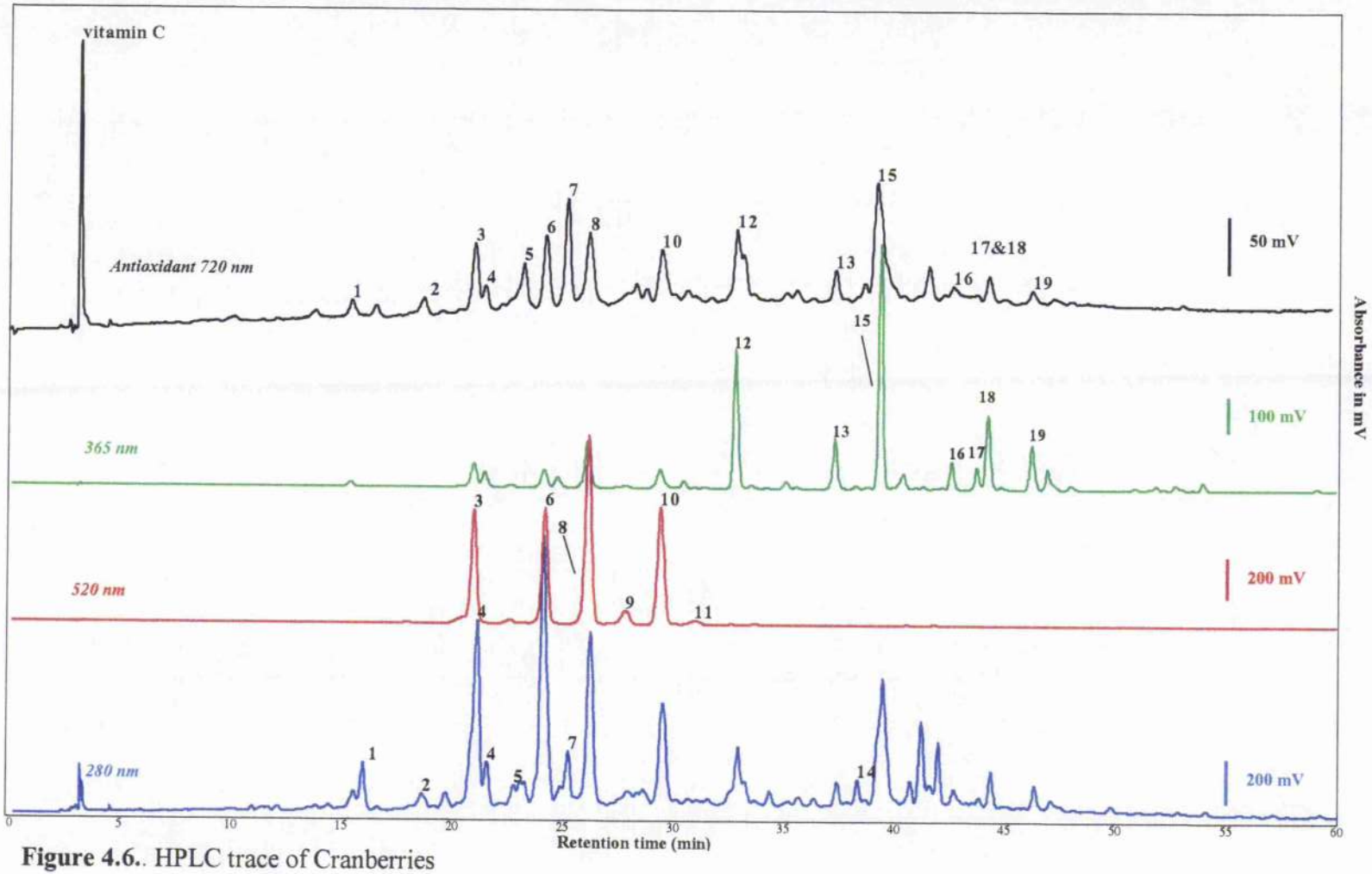


Figure 4.6.. HPLC trace of Cranberries

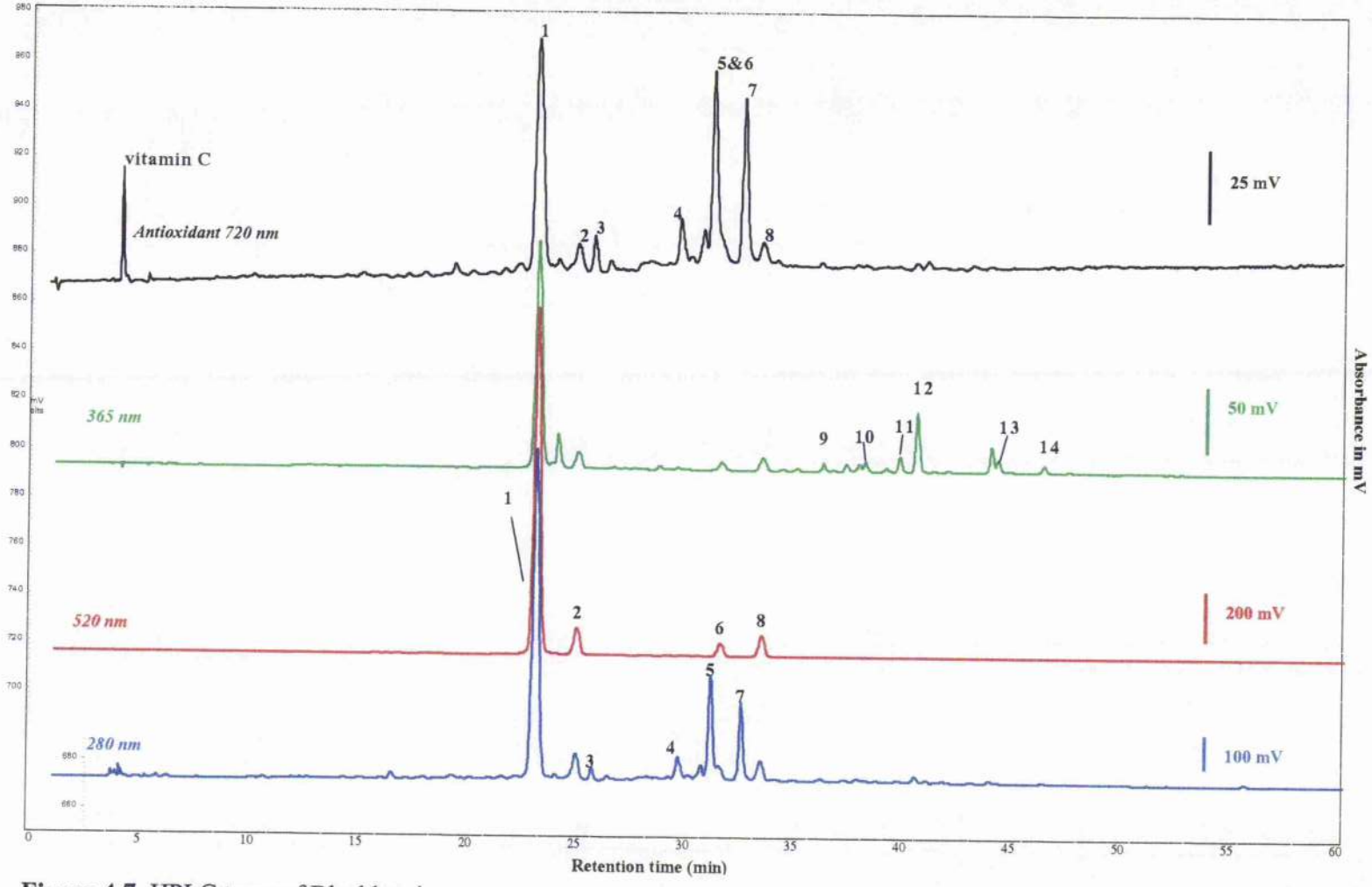


Figure 4.7. HPLC trace of Blackberries

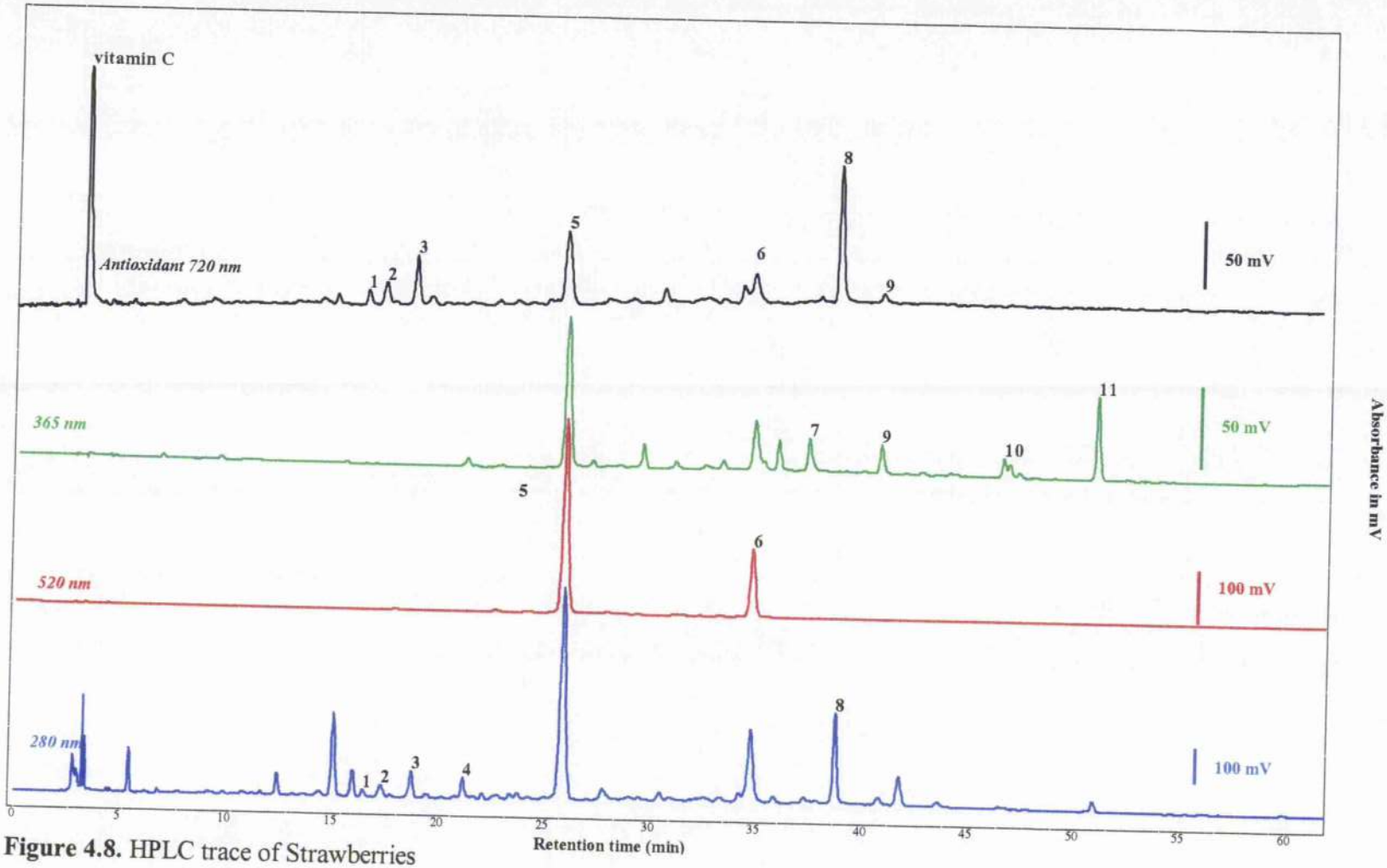


Figure 4.8. HPLC trace of Strawberries

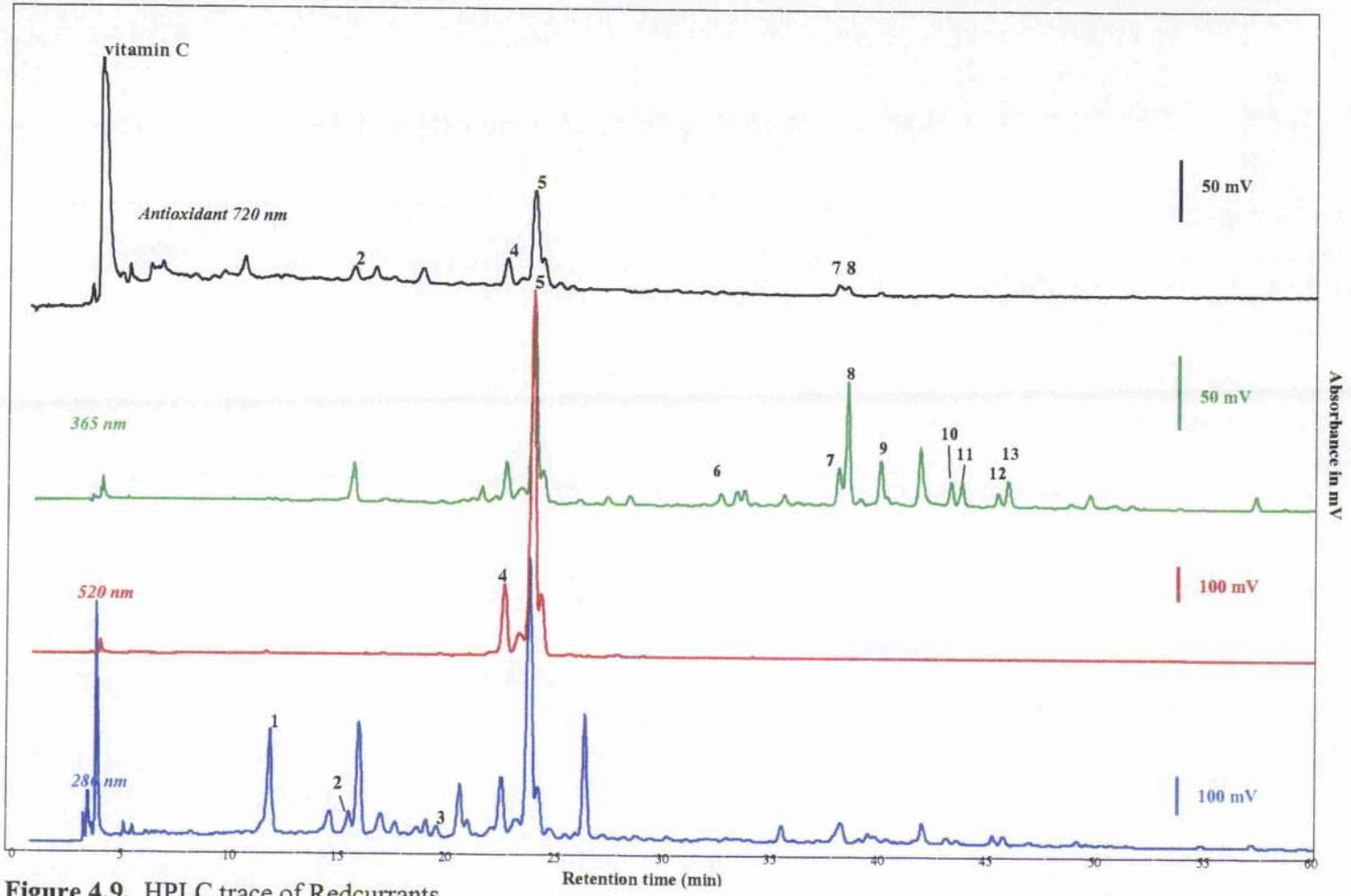


Figure 4.9.. HPLC trace of Redcurrants

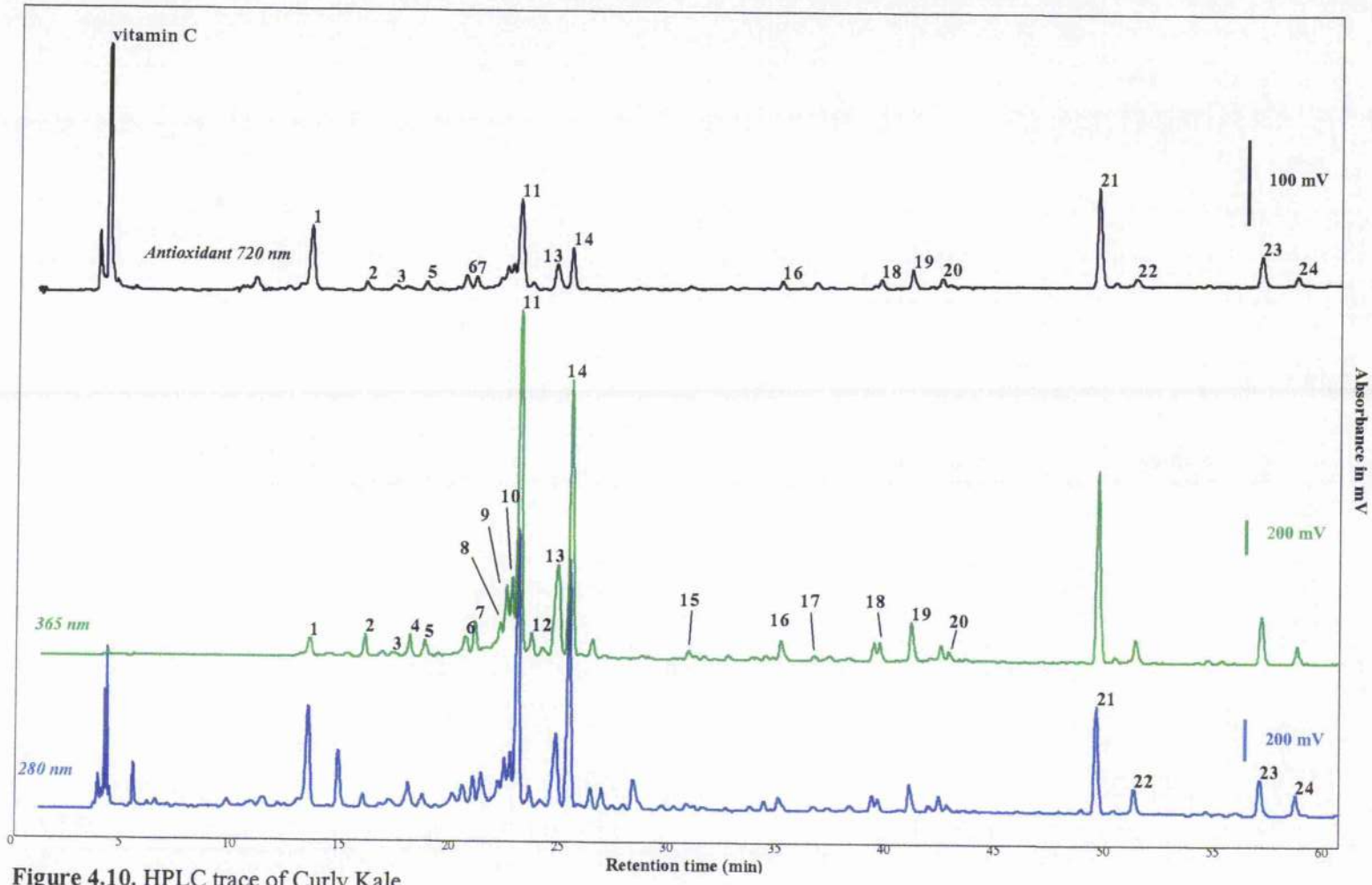


Figure 4.10. HPLC trace of Curly Kale

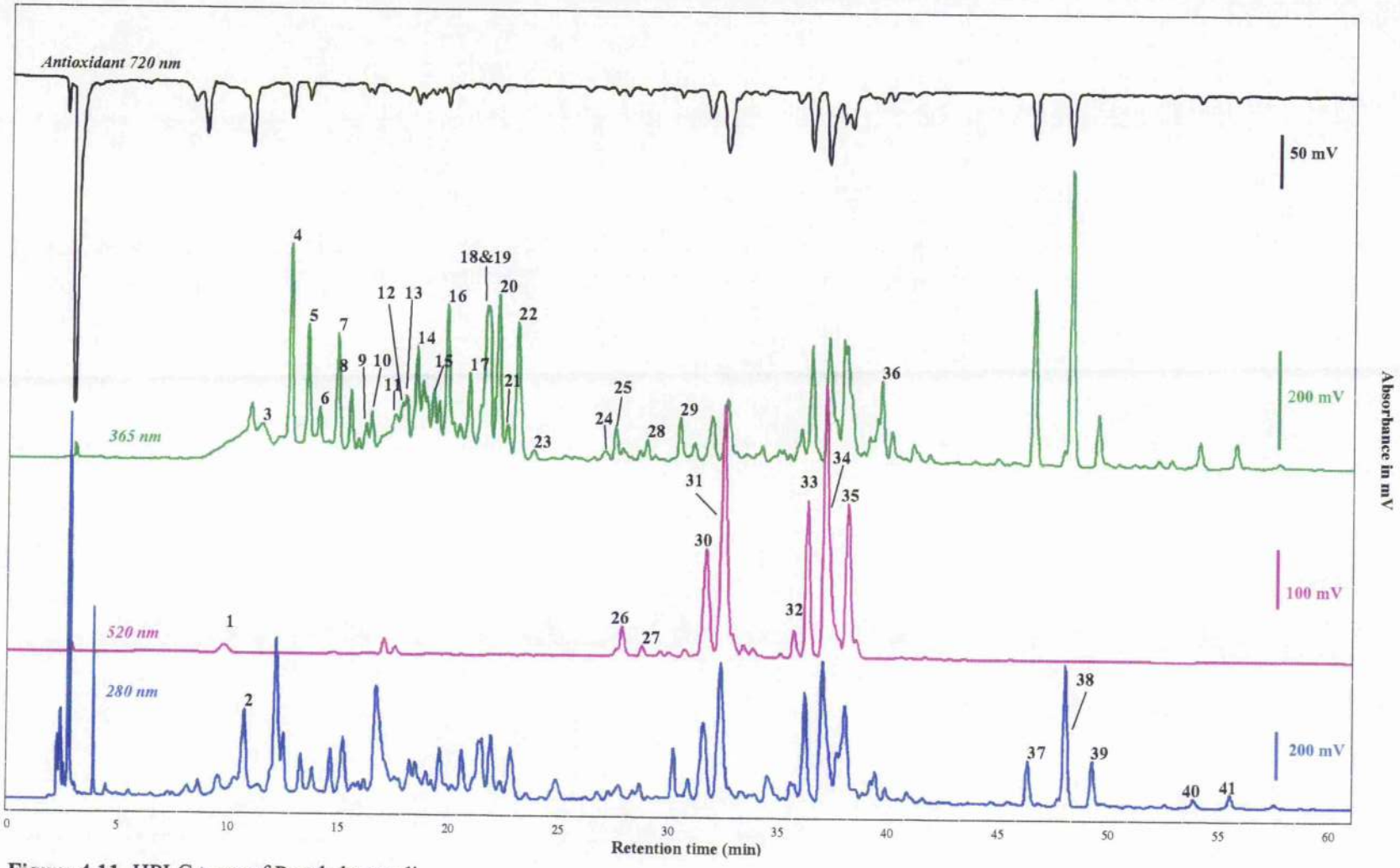


Figure 4.11. HPLC trace of Purple broccoli

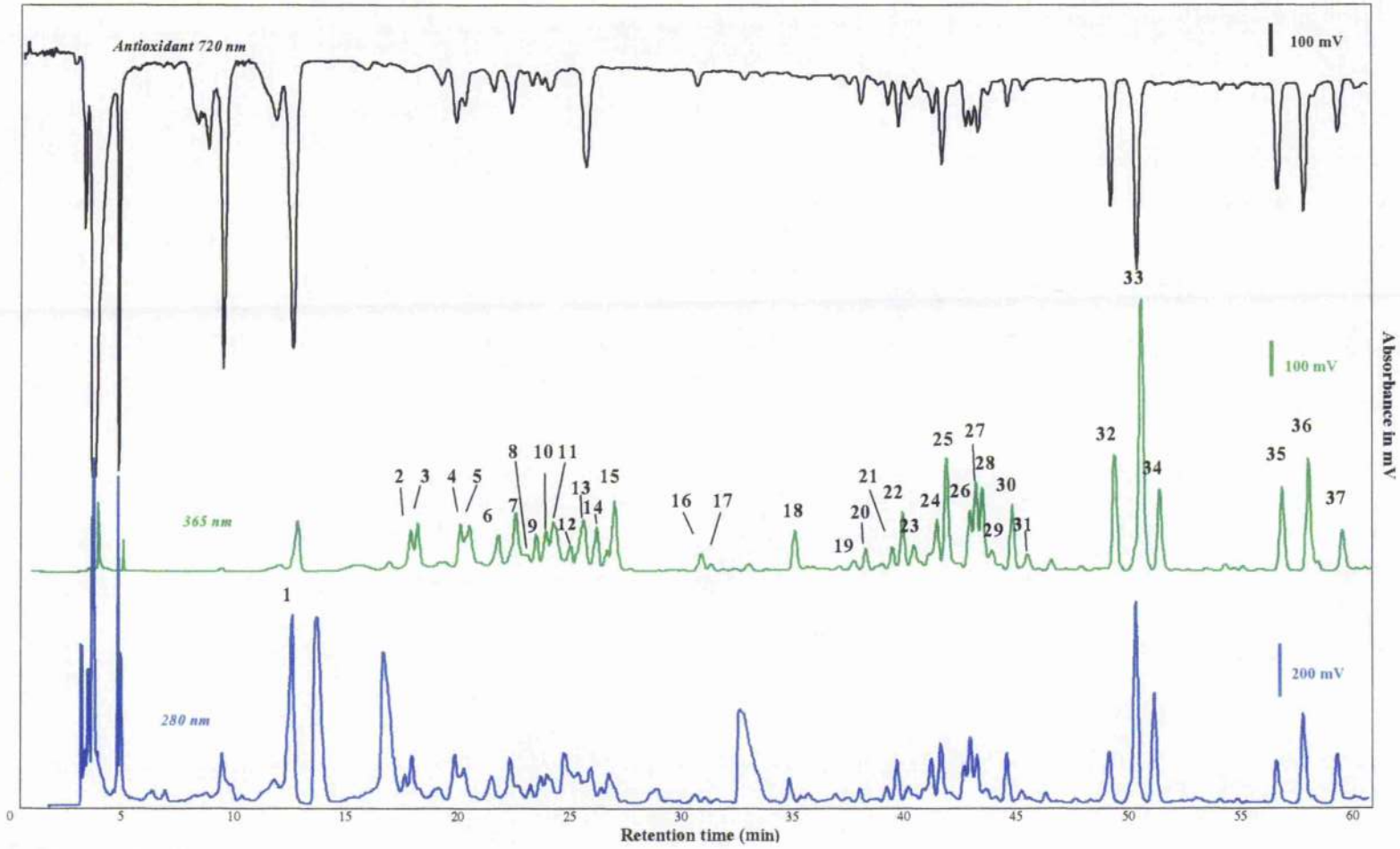


Figure 4.12. HPLC trace of broccoli

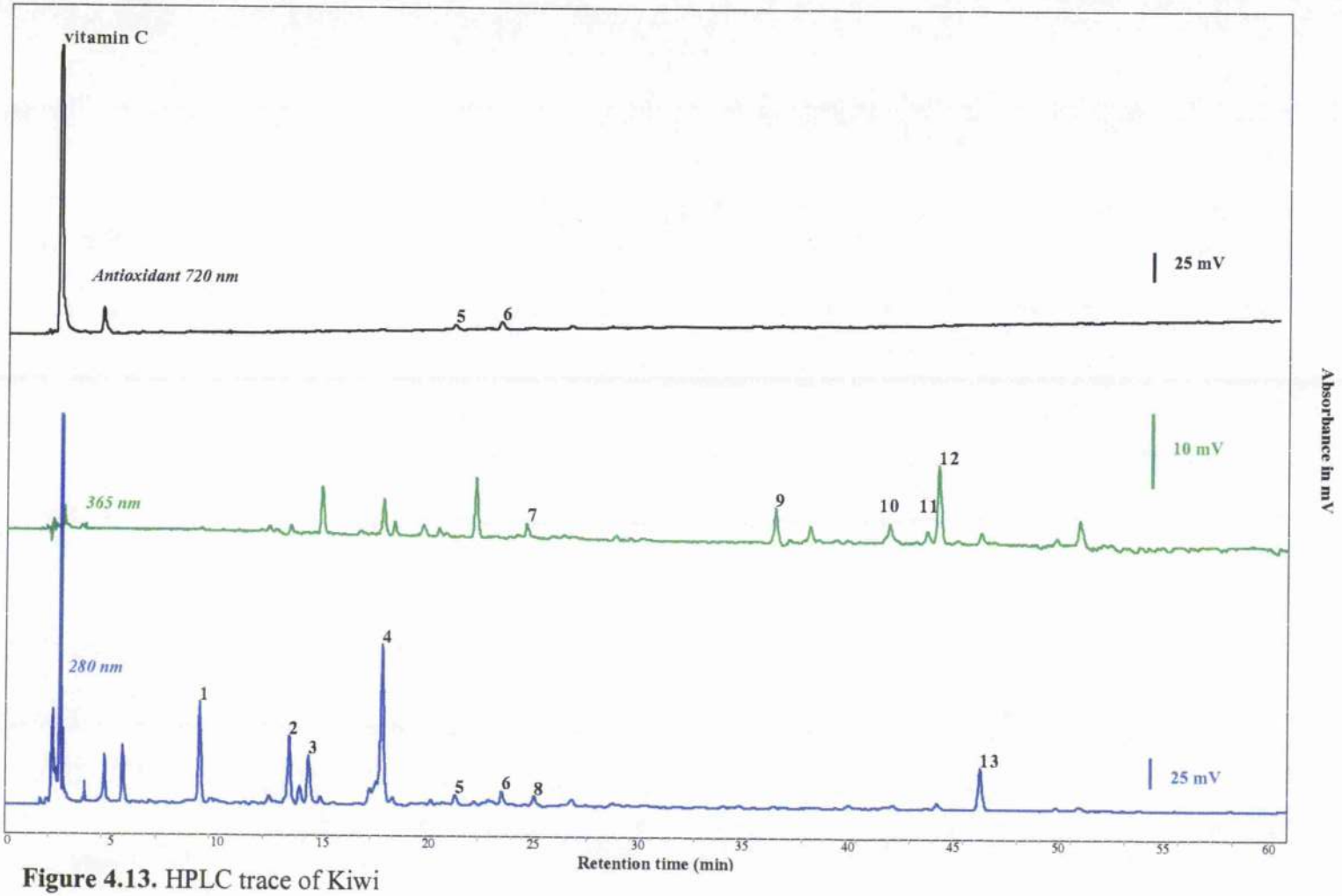


Figure 4.13. HPLC trace of Kiwi

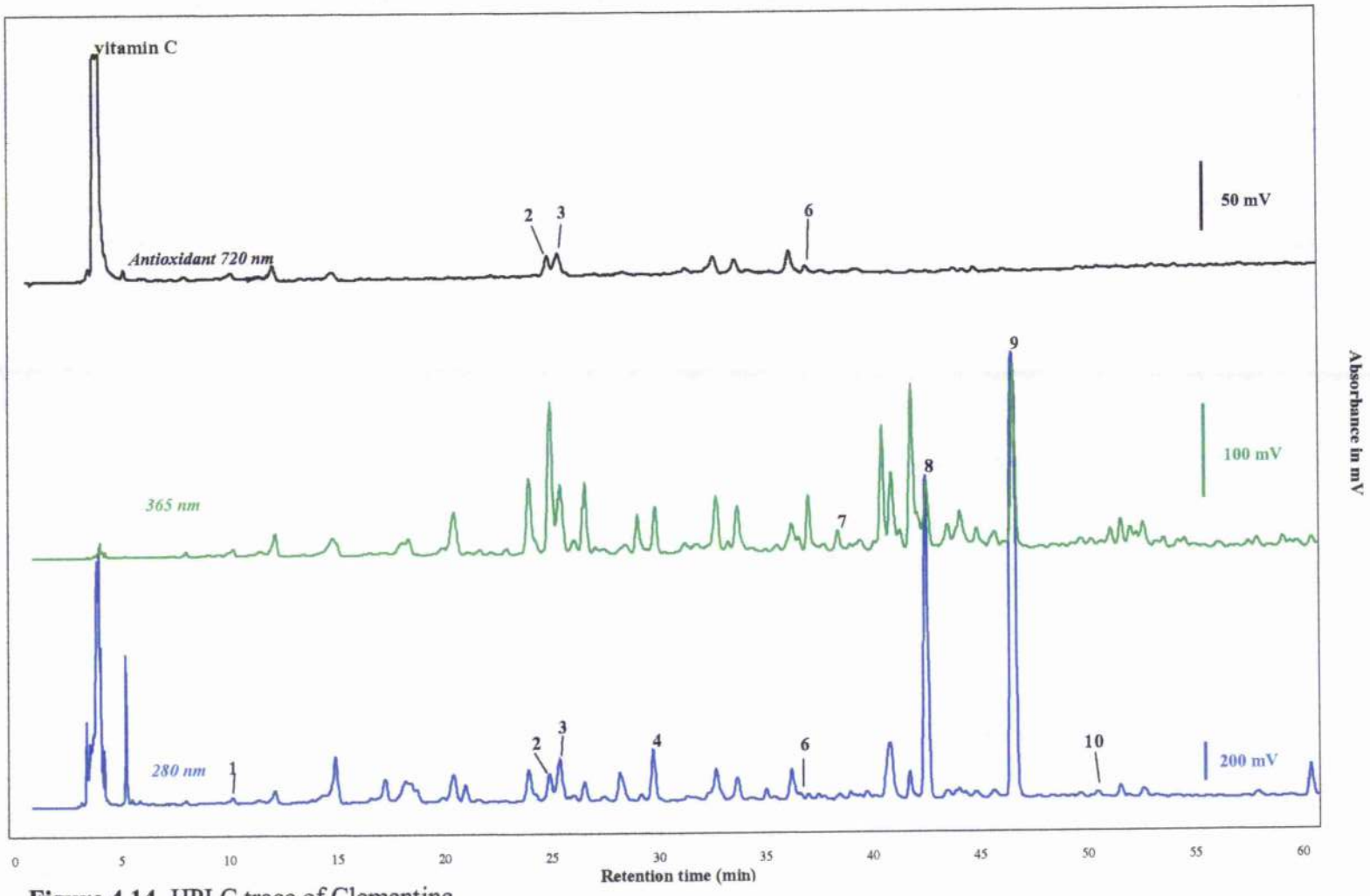


Figure 4.14. HPLC trace of Clementine

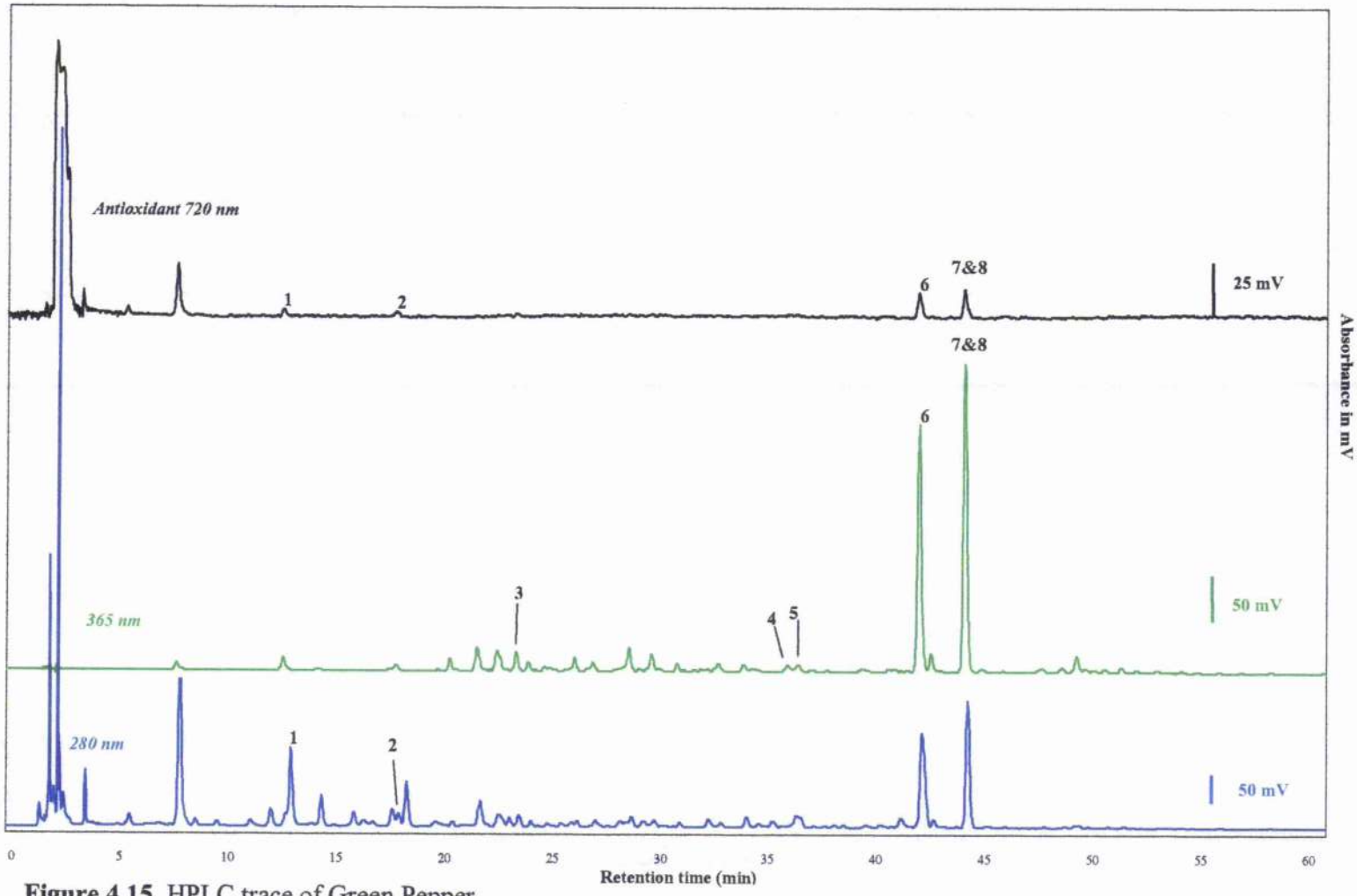


Figure 4.15. HPLC trace of Green Pepper

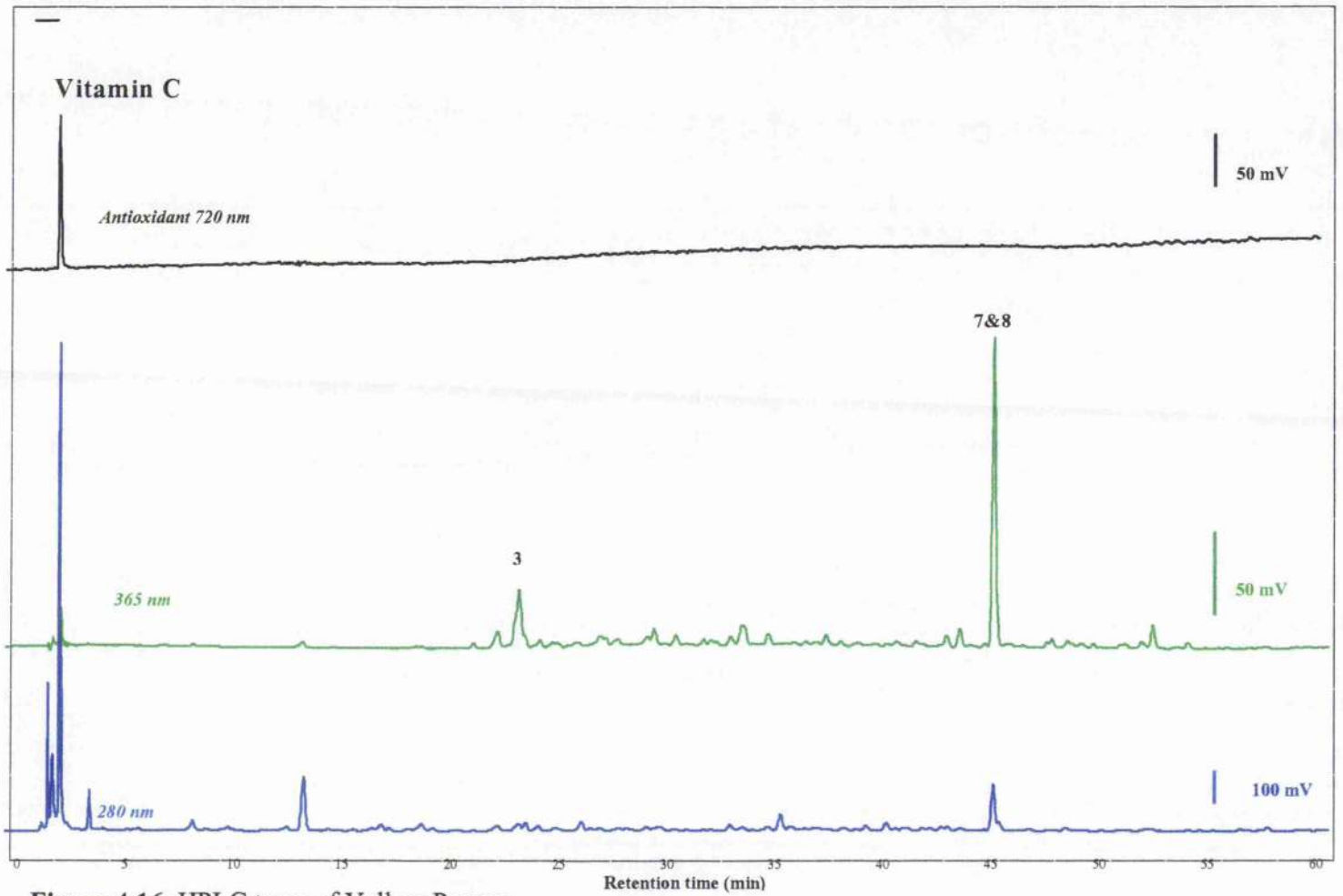


Figure 4.16. HPLC trace of Yellow Pepper

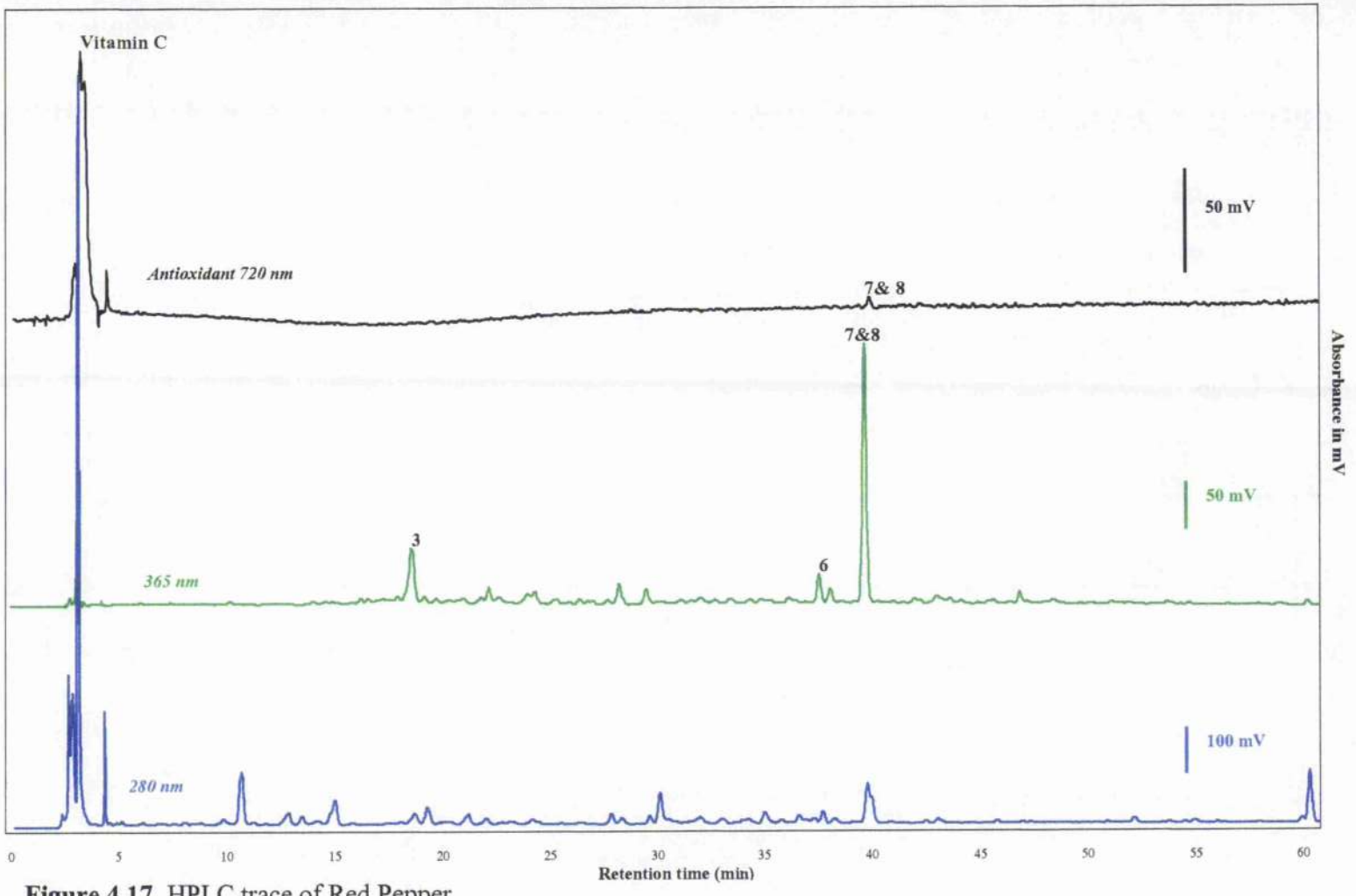


Figure 4.17. HPLC trace of Red Pepper

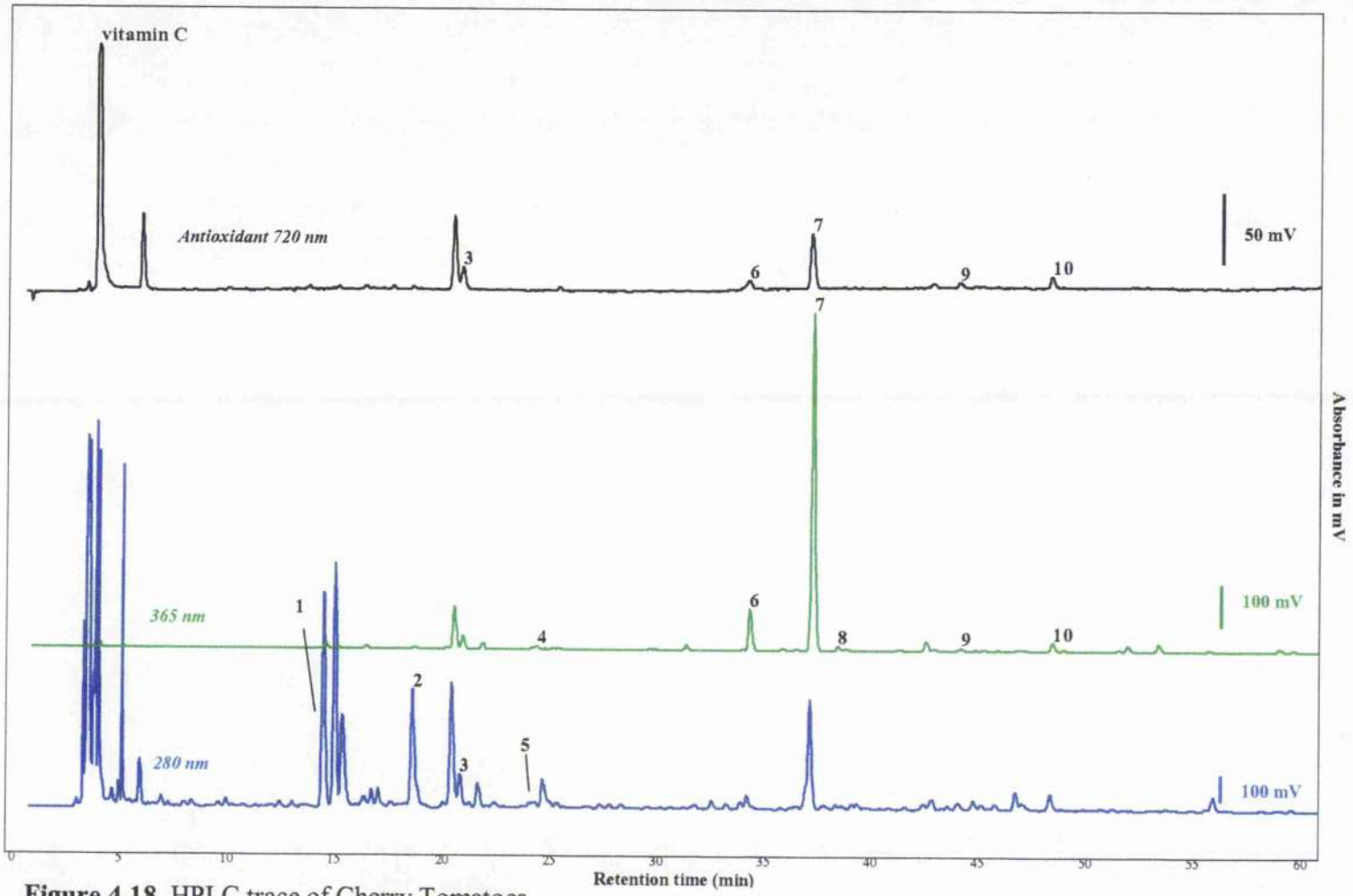


Figure 4.18. HPLC trace of Cherry Tomatoes

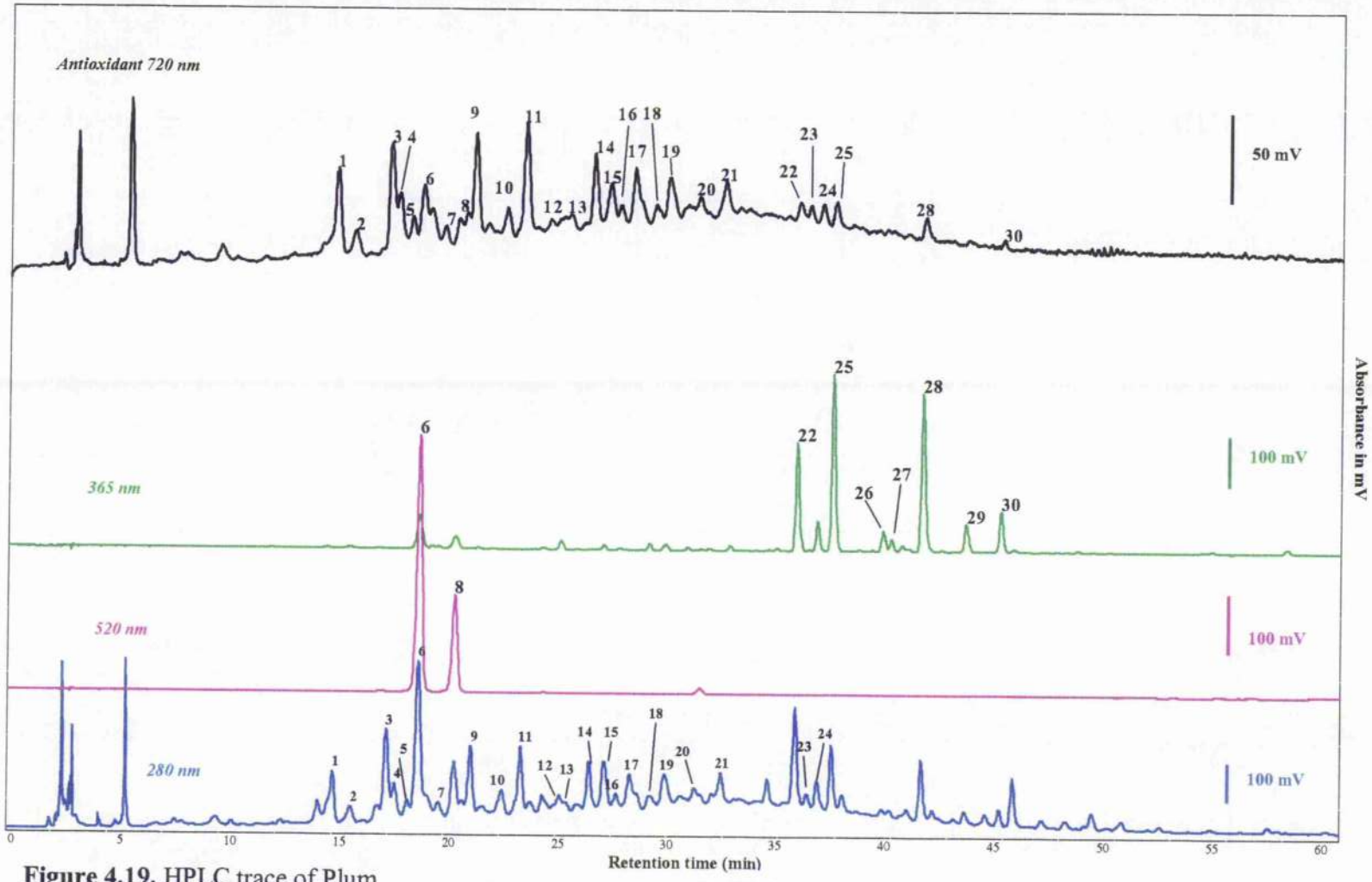


Figure 4.19. HPLC trace of Plum

Table 4.1. Summary of phenolic compounds detected in an extract of raspberries by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ *(m/z)*	MS^2 ions *(m/z)*	MS^3 ions *(m/z)*
1	17.9	cyanidin-3-sophoroside	280, 515	611*	287 [Cyan]($[M+H]^+$-Soph)	
2	20.0	cyanidin-3-(2^G-glucosylrutinoside)	280, 515	757*	611 ($[M+H]^+$-Rham), 287 [Cyan]($[M+H]^+$-Rham-Glc-Glc)	
		cyanidin-3-sambubioside		581*	287 ($[M+H]^+$-Samb)	
		cyanidin-3-glucoside		449*	287 [Cyan]($[M+H]^+$-Glc)	
3	21.0	pelargonidin-3-sophoroside	275, 500	595*	271 [Pel]($[M+H]^+$-Soph)	
4	22.5	cyanidin-3-rutinoside	280, 510	595*	449 (M^+-Rham), 287 [Cyan](M^+-Rham-Glc)	
		cyanidin-3-xylosylrutinoside		727*	581 ($[M+H]^+$-Rham), 287 ($[M-H]^-$-Rham-Xyl-Glc)	
		(-)-epicatechin		289	245, 205, 179	
5	23.3	pelargonidin-3-glucoside	270, 500	433*	271 [Pel]($[M+H]^+$-Glc)	
		pelargonidin-3-(2^G-glucosylrutinoside)		741*	271 [Pel](M^+- Rham-Glc-Glc)	
		sanguiin H-10		[783][2] (1565)	1265 ($[M-H]^-$-HHDP), 1103 ($[M-H]^-$-HHDP-Glc), 933 ($[M-H]^-$-HHDP-Glc-galloyl), 631 ($[M-H]^-$-HHDP-Glc-galloyl-HHDP)	
6	29.1	lambertianin C	250	1401[2] (2801)	1869, 1567 (1869-HHDP), 1265 (1869-HHDP-HHDP), 1251, 935 (1869-HHDP-HDDP-Glc-galloyl), 633 (1869-HHDP-HHDP-Glc-galloyl-HHDP)	
7	30.5	sanguiin H-6	250	1869	1567 ($[M-H]^-$-HHDP), 1265 ($[M-H]^-$-HHDP-HHDP), 1235, 933 ($[M-H]^-$-HHDP-HHDP-Glc-galloyl), 631 ($[M-H]^-$-HHDP-HHDP-Glc-galloyl-HHDP)	
8	33.8	ellagic acid-pentose conjugate	255, 360	433	301 [HHDP]($[M-H]^-$-pent)	257
9	35.1	ellagic acid	250, 360	301	257	
10	37.2	quercetin-galactosylrhamnoside	255, 340	609	301 [Q]($[M-H]^-$-Gal-Rham)	
11	38.2	quercetin-3-rutinoside	255, 340	609	301 [Q]($[M-H]^-$-Glc-Rham)	
12	38.5	quercetin-3-galactoside	285, 355	463	301 [Q]($[M-H]^-$-Gal)	
13	39.4	quercetin-3-glucuronide	255, 340	477	301 [Q]($[M-H]^-$-GlcAC), 179	
14	44.0	methyl-ellagic acid pentose	255, 345	447	315 ($[M-H]^-$-Pent), 301 [HHDP]	
15	44.1	ellagic acid-4-acetylxyloside	255, 360	475	301 [HHDP]($[M-H]^-$-XylAc)	
16	47.8	ellagic acid-4-acetylarabinoside	255, 365	475	301 [HHDP]($[M-H]^-$-AraAc)	

Cyan, cyanidin; Pel, pelargonidin; Soph, sophorosyl; Samb, sambubiosyl; Q, quercetin; Glc, glucosyl; Gal, galactosyl; Rham, rhamnosyl; Pent, pentosyl; Xyl, xylosyl; XylAc, acetylxylosyl; AraAc, acetylarabinosyl; GlcUA, glucuronyl; HHDP, hexahydroxydiphenoyl; t_R, retention time

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.3. * positive ionisation molecular ion ($[M+H]^+$)

Table 4.2.. Summary of phenolic compounds detected in an extract of blueberries by HPLC with diode array and MS^3 detection[a]

peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ *m/z*	MS^2 ions (*m/z*)	MS^3 ions (*m/z*)
1	15.6	delphinidin-3-galactoside	275, 520	465*	303 [Del]([M+H]$^+$- Gal)	
3	18.9	5-caffeoylquinic acid	300, 325	353	191, 179	
2	19.2	cyanidin-3-galactoside	275, 520	449*	287 [Cyan]([M+H]$^+$- Gal)	
		delphinidin-3-arabinoside		435*	303 [Del]([M+H]$^+$- Arab)	
4	21.4	petunidin-3-galactoside	275, 520	479*	317 [Pet]([M−H]$^+$- Gal)	
5	22.5	cyanidin-3-arabinoside	280, 515	419*	287 [Cyan]([M+H]$^+$- Arab)	
6	24.7	petunidin-3-arabinoside	275, 520	449*	317 [Pet]([M−H]$^+$- Arab)	
		peonidin-3-galactoside		463*	301 [Peon]([M+H]$^+$- Gal)	
7	26.7	malvidin-3-galactoside	275, 520	493*	331 [Malv]([M+H]$^+$- Gal)	
8	27.6	malvidin-3-glucoside	275, 525	493*	331 [Malv]([M+H]$^+$- Glc)	
		delphinidin-6-acetyl-3-glucoside		507*	303 [Del]([M−H]$^-$- AcGlc)	
9	28.3	peonidin-3-arabinoside	275, 525	433*	301 [Peon]([M+H]$^+$- Arab)	
10	30.4	malvidin-3-arabinoside	275, 525	463*	331 [Malv]([M+H]$^+$- Arab)	
11	31.8	myricetin-3-galactoside	255, 355	479	317 [Myr]([M-H]$^-$-Gal)	
12	33.5	petunidin-6-acetyl-3-glucoside	275, 525	521*	317 [Pet]([M+H]$^+$- AcGlc)	
13	36.8	myricetin-pentose	255, 355	439	317 [Myr]([M-H]$^-$- pentose)	
		5-feruloylquinic acid	325	367	191, 179, 135	
14	38.7	malvidin-6-acetyl-3-glucoside	275, 530	535*	331 [Malv]([M+H]$^+$- AcGlc)	
15	39.1	quercetin-3-rutinoside	255, 355	609	301 [Q] ([M-H]$^-$-Rut)	179, 151
		quercetin-3-galactoside	255, 355	463	301 [Q] ([M-H]$^-$ -Gal)	
16	40.1	quercetin-3-glucoside	255, 355	463	301 [Q] ([M-H]$^-$ -Glc)	
16'	40.6	unknown	255, 355	493	330, 315, 271	
17	43.9	quercetin-3-arabinoside	255, 355	433	301 [Q] ([M-H]$^-$ -Arab), 179	271, 255, 179

Del, delphinidin; Glc, glucosyl; Cyan, cyanidin; Arab, arabinosyl; Pet, petunidin; Peon, peonidin; Malv, malvidin; Gal, galactosyl; AcGlc, acetylglucosyl; Myr, myricetin; Hex, hexosyl; Rut, rutinosyl; Q, quercetin; Pent, pentosyl; t_R, retention time

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.4 * positive ionisation molecular ion ($[M+H]^+$)

Table 4.3. Summary of phenolic compounds detected in an extract of blackcurrants by HPLC with diode array and MS^3 detection[a]

peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ *m/z*	MS^2 ions (*m/z*)	MS^3 ions (*m/z*)
1	13.1	caffeic acid-glucoside conjugate	330	341	179 [CafAc]([M-H]$^-$ - Hex)	
2	14.7	delphinidin-3-galactoside	275, 520	465*	303 [Del]([M+H]$^+$ - Gal)	
3	16.1	delphinidin-3-glucoside	275, 520	465*	303 [Del]([M+H]$^+$ - Glc)	
4	18.2	cyanidin-3-galactoside	275, 520	449*	287[Cyan]([M+H]$^+$ - Gal)	
		delphinidin-3-rutinoside		611*	465 ([M+H]$^+$ - Rham), 303 [Del]([M+H]$^+$ - Glc-Rham)	
		delphinidin-3-arabinoside		435*	303 [Del]([M+H]$^+$ - Arab)	
		5-caffeoylquinic acid		353	191,179	
5	19.6	cyanidin-3-glucoside	280, 515	449**	287[Cyan]([M+H]$^+$ - Glc)	
6	20.8	petunidin-3-galactoside	275, 520	479*	317 [Pet]([M+H]$^+$ - Gal)	
7	22.1	cyanidin-3-arabinoside	280, 515	419*	287[Cyan]([M+H]$^+$ - Arab)	
		cyanidin-3-rutinoside		595*	449 ([M+H]$^+$ - Rham), 287 [Cyan]([M+H]$^+$ - Glc-Rham)	
		petunidin-3-glucoside		479*	317 [Pet]([M+H]$^+$ - Glc)	
8	24.2	peonidin-3-galactoside	275, 520	463*	301 [Peon]([M+H]$^+$ - Gal)	
		petunidin-3-rutinoside		625*	479 ([M+H]$^+$ - Rham), 317 [Pet]([M+H]$^+$ - Rham-Glc)	
9	25.9	malvidin-3-galactoside	280, 520	493*	331 [Malv]([M+H]$^+$ - Gal)	
		peonidin-3-glucoside		463*	301 [Peon]([M+H]$^+$ - Glc)	
10	27.7	malvidin-3-glucoside	275, 525	493*	331 [Malv]([M+H]$^+$ - Glc)	
		peonidin-pentose		433*	301 [Peon]([M+H]$^+$ - Pent)	
		peonidin-3-rutinoside		609*	463 (M^+ - Rham), 301 ([Peon]([M+H]$^+$ - Glc-Rham)	
11	29.6	malvidin-3-arabinoside	275, 525	463*	331 [Malv]([M+H]$^+$ - Arab)	
12	31.6	myricetin-3-rutinoside	250, 355	625	317 [Myr]([M-H]$^-$ - Rut)	
13	32.2	myricetin-glucuronide	250, 355	493	317 [Myr]([M-H]$^-$ - GlcUA)	
14	35.5	myricetin-3-malonylglucoside	250, 355	521	317 [Myr]([M-H]$^-$ - Glc-(Mal-COOH))	
15	38.4	quercetin-3-rutinoside	255, 350	609	301 [Q]([M-H]$^-$ - Rut)	
16	38.7	quercetin-3-galactoside	250, 350	463	301 [Q]([M-H]$^-$ - Gal)	271, 255, 179
17	39.7	quercetin-3-glucuronide	255, 355	477	301 [Q]([M-H]$^-$ - GlcUA)	
		quercetin-3-glucoside	250, 350	463	301 [Q]([M-H]$^-$ - Glc)	
18	42.8	delphinidin-3-*O*-6″-coumaroylglucoside	280, 525	611*	303 [Del]([M+H]$^+$ - Coum-Glc)	
19	43.8	quercetin-3-malonylglucoside	250, 350	505	301 [Q]([M-H]$^-$ - Glc-(Mal-COOH))	271, 255, 179
20	44.6	kaempferol-3-rutinoside	265, 315	593	285 [K]([M-H]$^-$ - Rut)	
21	46.2	kaempferol-3-glucoside	250, 340	477	301 [K]([M-H]$^-$ - Glc)	
22	47.9	unknown	250, 360	507	345([M-H]$^-$ - Hex)	301, 316,255

CafAc, caffeic acid; Del, delphinidin; Hex, hexosyl; Gal, galactosyl; Glc, glucosyl; Rut, rutinosyl; Arab, arabinosyl; Cyan, cyanidin; Pet, petunidin; Peon, peonidin; Malv, malvidin; Myr, myricetin; GlcUA, glucuronyl; Pent, pentosyl; Mal, malonyl; Q, quercetin; Coum, coumaroyl; K, kaempferol; t_R, retention time

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.5 * positive ionisation molecular ion ([M+H]$^+$)

Table 4.4. Summary of phenolic compounds detected in an extract of cranberries by HPLC with diode array and MS^3 detection[a]

peak	t_R	Compound	Absorbance(nm)	$[M-H]^-$ *m/z*	MS^2 ions (*m/z*)	MS^3 ions (*m/z*)
		vitamine C				
1	13.1	procyanidin dimer	290	577	425, 289 [Cat] ([M-H]$^-$ - Cat)	
2	15.7	(+)-catechin	245, 280	289	245	
	16.9	caffeic acid hexose conjugate	290, 315	341	179 [CafAc]([M-H]$^-$ -Hex)	
3	18.3	cyanidin-3-galactoside	280, 315, 515	449*	287 [Cyan] ([M+H]$^+$-Gal)	
		p-coumaric acid hexose conjugate		325	163 [CoumAc]([M-H]$^-$ -Hex),145,187	
4	18.7	5-caffeoylquinic acid	325	353	191, 179	
5	20.2	procyanidin dimer	245, 280	577	425, 289 [Cat] ([M-H]$^-$ - Cat)	
	21.9	cyanidin-3-arabinoside	280, 515	419*	287 [Cyan] ([M+H]$^+$ - Arab)	
6		procyanidin hexamer		1725	1437([M-H]$^-$-Cat), 1149([M-H]$^-$-Cat-Cat),861, ([M-H]$^-$ - Cat-Cat-Cat), 573([M-H]$^-$ -Cat-Cat-Cat-Cat)	
7	22.2	(-)-epicatechin	280	289	245	
8	24.2	peonidin-3-galactoside	280, 515	463*	301 [Peon] ([M+H]$^+$- Gal)	
9	26.2	malvidin-3-glucoside	280, 520	593*	331 [Malv] ([M+H]$^+$- Glc)	
		procyanidin polymer		867	577([M-H]$^-$ - Cat), 289([M-H]$^-$ -Cat-Cat)	
10	27.9	peonidin-3-arabinoside	280, 515	433*	301 [Peon] ([M+H]$^+$ - Arab)	
11	29.9	malvidin-3-arabinoside	280, 520	463*	331 [Malv] ([M+H]$^+$ - Arab)	
12	31.3	myricetin-3-galactoside	265, 355	479	317 [Myr] ([M-H]$^-$ -Gal)	271, 179, 151
		procyanidin dimer		575	449, 289([M-H]$^-$-Cat)	
13	36.2	myricetin-3-arabinoside	255, 355	449	317 [Myr]([M-H]$^-$ - Arab)	271, 179, 151
		procyanidin polymer		863	1151,575,779	
14	37.7	procyanidin dimer		577	425, 289[Cat] ([M-H]$^-$ - Cat)	
15	38.6	quercetin-3-galactoside	255, 355	463	301 [Q] ([M-H]$^-$ - Gal)	271, 255, 179
		procyanidin trimer		865	577([M-H]$^-$-Cat), 289([M-H]$^-$-Cat-Cat)	
16	42.2	quercetin-3-xylopyranoside	250, 355	433	301[Q] ([M-H]$^-$ - Xyl)	271,255,179
17	43.4	quercetin-3-arabinopyranoside	270, 350	433	301[Q] ([M-H]$^-$ - Arab)	
18	44.0	quercetin-3-arabinofuranoside	270, 350	433	301[Q] ([M-H]$^-$ - Arab)	271,255,179
19	46.5	quercetin-3-rhamnoside	250, 345	447	301 [Q] ([M-H]$^-$ - Rham)	271,255,179

Cat, catechin; CafAc, caffeic acid; CoumAc, *p*-coumaric acid; Cyan, cyanidin; Mal, malvidin; Peon, peonidin; Myr, myricetin; Q, quercetin; Arab, arabinosyl; Glc, glucosyl; Gal, galactosyl; Rham, rhamnosyl; Pent, pentosyl; Hex, hexose; t_R, retention time

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.6 * positive ionisation molecular ion ($[M+H]^+$)

Table 4.5. Summary of phenolic compounds detected in an extract of blackberries by HPLC with diode array and MS^3 detection[a]

peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ *m/z*	MS^2 ions (*m/z*)	MS^3 ions (*m/z*)
1	19.5	cyanidin-3-glucoside	275, 505	449*	287 [Cyan] ($[M+H]^+$ - Glc)	
2	22.0	cyanidin-3-rutinoside	280, 515	595*	449($[M-H]^+$ - Rham), 287 [Cyan] ($[M+H]^+$ - Glc-Rham)	
3	23.5	sanguiin H-10	250	[783]2 (1566)	1265 ($[M-H]^-$-HHDP), 1104($[M-H]^-$-HHDP-Glc), 933($[M-H]^-$ - HHDP-Glc-galloyl), 633($[M-H]^-$-HHDP-HHDP-Glc-galloyl)	
4	27.3	sanguiin	250	1870	1567($[M-H]^-$-HHDP), 1265($[M-H]^-$HHDP-HHDP), 933 ($[M-H]^-$ 2HHDP-Glc-galloyl), 631($[M-H]^-$ - HHDP-HHDP-HHDP-Glc-galloyl)	
5	28.8	Lambertianin C	250	1401	1869,1568(1869-HHDP), 1251, 935 (1869-HHDP-HHDP-Glc-galloyl), 633(1869-HHDP-HHDP-HHDP-Glc-galloyl)	
6	29.6	cyanidin-malonylglucoside	280, 515	535*	287[Cyan]($[M+H]^+$ - MalGlc)	
7	30.2	sanguiin H-6	250	1870	1567($[M-H]^-$ -HHDP), 1235, 933($[M-H]^-$ - HHDP-HHDP-Glc-galloyl), 631($[M-H]^-$ - HHDP-HHDP-HHDP-Glc-galloyl), 613	
8	31.8	cyanidin-rutinoside	280, 515	593*	449 ($[M+H]^+$ - Rham), 287 [Cyan] (M^+ - Glc-Rham)	
	33.5	ellagic acid-pentose conjugate	255, 360	433	301[HDDP]($[M-H]^-$ -Pent)	257
9	35.3	unknown ellagic acid conjugate	255, 365	769	463($[M-H]^-$ -306),505, 301 [HHDP]($[M-H]^-$ -306 -Hex)	257, 230
10	36.8	quercetin-rutinoside	255, 355	609	301 [Q] ($[M-H]^-$ - Rut), 179	
11	38.2	quercetin-3-galactoside	255, 350	463	301 [Q] ($[M-H]^-$ - Gal), 179	
12	39.1	quercetin-3-glucuronide	255, 355	477	301 [Q] ($[M-H]^-$ - GlcUA)	179, 151, 257
		quercetin-3-glucoside	355	463	301 [Q] ($[M-H]^-$ - Glc)	
13	43.2	quercetin-malonate-hexoside	255-355	505	463($[M-H]^-$ -Mal-(COOH), 301 [Q] ($[M-H]^-$ - Hex- Mal-(COOH)), 179	255, 179
14	45.8	kaempferol-glucuronide	265, 345	461	285 [K] ($[M-H]^-$ GlcUA)	

Cyan, cyanidin, Glc, glucosyl; Rham, rhamnosyl; MalGlc, malonylglucosyl; Q, quercetin; K, kaempferol; HHDP, hexahydroxydiphenoyl; Pent, pentose; Rut, rutinosyl; GlcUA. Glucuronyl; Hex, hexose; t_R, retention time

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.7 * positive ionisation molecular ion ($[M+H]^+$)

Table 4.6. Summary of phenolic compounds detected in an extract of strawberries by HPLC with diode array and MS^3 detection[a]

peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ *m/z*	MS^2 ions (*m/z*)	MS^3 ions (*m/z*)
1	13.3	procyanidin dimer	280	577	289 [Cat]([M-H]$^-$ - Cat)	
2	14.3	procyanidin dimer	245, 280	577	289 [Cat]([M-H]$^-$ - Cat)	
3	15.7	procyanidin trimer	280	865	289 [Cat]([M-H]$^-$ -Cat-Cat)	
4	18.4	*p*-coumaric-hexose	315	325	163 [pCoum]([M-H]$^-$ -Hex)	
5	23.5	pelargonidin-3-glucoside	275, 430, 500	433*	271 [Pel]$^+$([M+H]$^+$ - Glc)	
6	33.6	pelargonidin-3-*O*-(6-*O*-malonylglucoside)	275, 430, 500	519*	271 [Pel]$^+$([M+H]$^+$ - MalGlc)	
7	34.9	ellagic acid rhamnoside	250, 375	447	301 [HHDP]([M-H]$^-$ - Rham)	257
8	37.9	sanguiin	250	1870	1567([M-H]$^-$-HHDP), 1265([M-H]$^-$ -HHDP-HHDP), 935([M-H]$^-$ - HHDP-HHDP-Glc-galloyl), 633 ([M-H]$^-$ -HHDP-HHDP-HHDP-Glc-galloy)	
9	39.5	quercetin-3-glucuronide	265, 355	477	301 [Q]([M-H]$^-$ -GlcUA)	179
10	46.0	kaempferol-3-glucoside	265, 340	447	285 [K]([M-H]$^-$ -Glc)	
11	50.9	kaempferol hexoside-malonate	265, 345	489	285[K]([M-H]$^-$ - Hex-(Mal-COOH))	255, 227, 135

Cat, catechin; pCoum, p-coumaric acid; Hex, hexosyl; Pel, pelargonidin; Glc, glucosyl; MalGlc, malonylglucosyl; HHDP, hexahydroxydiphenoyl; Rham, rhamnosyl; Q, quercetin; GlcUA, glucuronyl; K, kaempferol; Mal, malonyl; t_R, retention time

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.8 * positive ionisation molecular ion ($[M+H]^+$)

Table 4.7.. Summary of phenolic compounds detected in an extract of redcurrants by HPLC with diode array and MS^3 detection[a]

peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ *m/z*	MS^2 ions (*m/z*)	MS^3 ions (*m/z*)
1	9.2	4-hydroxy-benzoic acid-hexose	260	299	137 [HbenzAc][($[M-H]^-$ - Hex)	
2	11.2	caffeic acid-hexose conjugate	330	341	179 [CafAc]($[M-H]^-$ -Hex)	
3	16.1	caffeic acid-hexose conjugate	330	341	179 [CafAc]($[M-H]^-$ -Hex)	
4	20.3	cyanidin-3-sambubioside	280, 515	581*	287[Cyan]($[M+H]^+$-Samb)	
5	22.0	cyanidin-3-rutinoside	280, 515	595*	449($[M+H]^+$- Rham), 287[Cyan]($[M+H]^+$- Glc-Rham)	
		cyanidin-3-xylosyl-rutinoside		727*	581($[M+H]^+$- Rham), 287 [Cyan]($[M+H]^+$- Xyl-Rut)	
6	31.4	myricetin-rutinoside	255, 355	625	317 [Myr]($[M-H]^-$ - Rut)	
7	37.2	myricetin-rhamnoside	255, 330	463	317 [Myr]($[M-H]^-$ - Rham)	
8	38.1	quercetin-3-rutinoside	255, 355	609	301 [Q]($[M-H]^-$ - Rut)	271, 179, 255
9	39.6	quercetin-3-glucoside	255, 355	463	301 [Q]($[M-H]^-$ - Glc),	271, 179, 255
10	43.4	quercetin-hexoside-malonate	255, 345	505	463($[M-H]^-$ - Rut), 301 [Q]($[M-H]^-$ - Hex-(Mal-COOH)), 179	
11	44.4	kaempferol-rutinoside	255, 345	593	285 [K]($[M-H]^-$ - Rut)	
12	46.2	kaempferol-3-glucoside	255, 340	447	285[K]($[M-H]^-$ - Glc)	

HbenzAc, 4-hydroxybenxoic acid; Hex, hexosyl; CafAc, Caffeic acid; Cyan, cyanidin; Samb, sambubiosyl; Rut, rutinosyl; Xyl, xylosyl; Myr, Myricetin; Rham, rhamnosyl; Q, quercetin; Glc, glucosyl; Mal, malonyl; K, kaempferol; t_R, retention time

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.9 * positive ionisation molecular ion ($[M+H]^+$)

Table 4.8. Summary of phenolic compounds detected in an extract of curly kale by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	λmax	$[M-H]^-$ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
1	11.2	quercetin-triglucoside conjugate	325	787	625 ([M-H]$^-$-Hex), 463([M-H]$^-$-Hex-Caf), 301[Q] ([M-H]$^-$-Hex-Caf-Hex)	
		3-caffeoylquinic acid	325	353	191, 179	
2	13.2	quercetin-3-diglucoside-7-glucoside	355	787	625 ([M-H]$^-$-Glc), 463([M-H]$^-$-Glc-Glc),301[Q]([M-H]$^-$ -Glc-Glc-Glc)	
3	14.5	kaempferol conjugate (dihexoside + unidentified 198 *amu* group)	320	807	609([M-H]$^-$ -198), 447([M-H]$^-$-Glc-198)	285[K]([M-H]$^-$-198-Glc-Glc)
4	15.3	kaempferol-3-diglucoside-7-glucoside	345	771	609([M-H]$^-$ -Glc), 285[K]([M-H]$^-$-Glc-Glc-Glc)	429, 285[K]([M-H]$^-$ -Glc-Glc-Glc)
5	16.1	quercetin conjugate (trihexoside – unidentified 192 *amu* group)	250-335	979	817([M-H]$^-$-Hex), -787([M-H]$^-$-192), -625([M-H]$^-$-Hex-192)	462([M-H]$^-$ -Hex-192-Hex)- 301[Q]([M-H]$^-$ - Hex-192-Hex-Hex)
6	17.9	kaempferol conjugate (dihexoside + unidentified 192 *amu* group)	330	963	801([M-H]$^-$-Hex)	609 ([M-H]$^-$ -Hex-192) –285[K]([M-H]$^-$ -Hex-192-Hex-Hex)
7	18.2	quercetin-3-feruloyldiglucoside-7-glucoside	335	963	801([M-H]$^-$-Glc),787([M-H]$^-$-Fer), 625([M-H]$^-$-Fer-Glc), 445	
8	19.2	quercetin-sinapoyl-tetraglucoside	340	1155	993([M-H]$^-$-Glc),949([M-H]$^-$-Sin),787([M-H]$^-$-Sin-Glc)	
9	19.5	quercetin –sinapoyl-pentaglucoside	340	1317	1111([M-H]$^-$-Sin), 993([M-H]$^-$-Glc-Glc),787([M-H]$^-$-Glc-Glc-Sin), 463([M-H]$^-$-Glc-Glc- Sin-Glc-Glc)	
10	19.9	kaempferol conjugate (kaempferol-sinapoyl-triglucoside +unidentified 198 *amu* group)	340	1177	853([M-H]$^-$-Hex-Hex), 647([M-H]$^-$-Hex-Hex-Syn),607,365,449([M-H]$^-$-Hex-Hex-Syn-198)	
11	20.2	quercetin-3-sinapoyldiglucoside-7-glucoside	340	993	831([M-H]$^-$-Hex),787([M-H]$^-$-Sin),625([M-H]$^-$-Sin-Hex),445	
12	20.7	quercetin-feruloyl-triglucoside	335	963	801([M-H]$^-$-Glc)-787([M-H]$^-$-Fer),625([M-H]$^-$-Glc-Fer)	
13	21.8	kaempferol-3-sinapoyltriglucoside-7-glucoside	330	1139	977([M-H]$^-$-Glc),771([M-H]$^-$-Glc-Sin),609([M-H]$^-$-Glc-Sin-Glc)	771, 429, 285 [K]
14	22.4	kaempferol-sinapoyl-triglucoside	330	977	815([M-H]$^-$-Glc),609([M-H]$^-$-Glc-Sin),447([M-H]$^-$ Glc-Sin-Glc)	
15	27.5	kaempferol-sinapoyl-tetraglucoside	345	1139	815([M-H]$^-$-Glc-Glc),609([M-H]$^-$-Glc-Glc-Sin)	447([M-H]$^-$ -Glc-Sin-Glc), 285[K]([M-H]$^-$-Glc-Sin-Glc-Glc)
16	31.6	quercetin-3-sinapoyldiglucoside	335	831	625([M-H]$^-$ -Sin), 445, 300[Q]([M-H]$^-$ -Sin-Glc-Glc)	
17	33.1	quercetin-3-rutinoside	330	609	301[Q]([M-H]$^-$-Glc-Rham)	
18	36	quercetin-3-disinapoyltriglucoside-7-glucoside	335	1522	1199([M-H]$^-$-Glc-Glc),993([M-H]$^-$-Glc-Glc-Sin),787([M-H]$^-$-Glc-Glc-Sin-Sin)	
19	37.3	quercetin –disinapoyl-tetraglucoside	335	1361	1199([M-H]$^-$ -Glc), 1155([M-H]$^-$-Sin),993([M-H]$^-$-Sin-Glc), 787([M-H]$^-$-Sin-Glc-Sin),	
20	39	kaempferol-3-disinapoyltriglucoside-7-glucoside	330	1345	1183([M-H]$^-$-Glc),977([M-H]$^-$-Sin)	
21	45.6	1,2-disinapoylglucoside	330	753	529([M-H]$^-$-SinAc), 289, 223	
22	47.3	1-sinapoyl-2-feruloyldiglucoside	330-245	725	501([M-H]$^-$-SinAc)	
23	53	1,2,2'-trisinapoyldiglucoside	325	961	737([M-H]$^-$-SinAc),531([M-H]$^-$-SinAc-Sin)	
24	54.6	1,2'-disinapoyl-2-feruloyldiglucoside	325	931	707([M-H]$^-$-SinAc),501([M-H]$^-$-SinAc-Sin)	481

Q, quercetin; K, kaempferol; Hex, hexosyl; Caf, caffeoyl; Glc, glucosyl; Fer, feruloyl; Sin, sinapoyl; Rham, rhamnosyl; SinAc, sinapic acid

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.16.

Table 4.9. Summary of phenolic compounds detected in an extract of purple broccoli by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	λmax	$[M-H]^-$ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
			335,			
1	10.6	cyanidin-3-diglucoside-5-glucoside [c]	515	773*	611([M+H]$^+$ -Glc), 449 ([M+H]$^+$ -Glc-Glc), 287[Cyan]([M+H]$^+$ -Glc-Glc-Glc)	
2	11.2	3-caffeoylquinic acid	325	353	191, 179	
3	12.9	quercetin-trihexoside	355	787	625 ([M-H]$^-$ -Hex), 301[Q]([M-H]$^-$ -Hex-Hex-Hex)	
4	13.5	quercetin-tetrahexoside	355	949	625([M-H]$^-$ -Hex-Hex), 445, 301[Q]([M-H]$^-$ -Hex-Hex-Hex-Hex-Hex)	445-301-271
5	14.3	quercetin-tetrahexoside	350	949	787([M-H]$^-$ -Hex) -625([M-H]$^-$ -Hex-Hex)	625, 463([M-H]$^-$ -Hex-Hex-Hex) 301[Q]([M-H]$^-$ -Hex-Hex-Hex-Hex)
6	14.8	kaempferol conjugate (dihexoside+unknown 198 *amu* group)	320	807	609([M-H]$^-$ -198), 447([M-H]$^-$ -198-Hex), 285[K]([M-H]$^-$ -198-Hex-Hex)	285,
			325,			
7	15.6	kampferol-sinapoyl-diglucoside	340	816	654([M-H]$^-$ -Hex), 609([M-H]$^-$ -Sin), 285[K]([M-H]$^-$ -Hex-Hex-Sin)	255-216
8	16.2	kaempferol conjugate (trihexoside+unknown 198 *amu* group)	345	969	903, 609([M-H]$^-$ -198-Hex)	447([M-H]$^-$ -198-Hex-Hex), 285[K]([M-H]$^-$ -198-Hex-Hex-Hex)
9	16.7	quercetin pentahexoside	335	1111	949([M-H]$^-$ -Glc), 787([M-H]$^-$ -Glc-Glc), 625([M-H]$^-$ -Glc-Glc-Glc)	787, 625, 301[Q]([M-H]$^-$ -Glc-Glc-Glc-Glc-Glc)
10	17.1	quercetin-hexahexoside	335	1273	1111([M-H]$^-$ -Hex), 949([M-H]$^-$ -Hex-Hex), 787([M-H]$^-$ -Hex-Hex-Hex), 625([M-H]$^-$ -Hex-Hex-Hex-Hex-Hex)	
11	18.2	kaempferol conjugate(pentahexoside+unknown 192 *amu* group)	325	1287	963([M-H]$^-$ -Hex-Hex), 771([M-H]$^-$ -Hex-192), 753, 429	
12	18.3	kaempferol conjugate (tetrahexoside+unknown 192 *amu* group)	335	1125	801([M-H]$^-$ -Hex-Hex). 609([M-H]$^-$ -Hex-Hex-192) -	609, 285[K]([M-H]$^-$ -Hex-Hex-Hex-Hex-192)
13	18.5	quercetin-trihexoside	355	787	625([M-H]$^-$ -Hex), 463([M-H]$^-$ -Hex-Hex), 301[Q]([M-H]$^-$ -Hex-Hex-Hex)	
14	19.0	quercetin-tetrahexoside	350	949	625([M-H]$^-$ -Hex-Hex)-301[Q]([M-H]$^-$ -Hex-Hex-Hex-Hex)	
15	19.2	kaempferol-pentahexosice	335	1095	933([M-H]$^-$ -Glc), 771([M-H]$^-$ -Glc-Glc), 609([M-H]$^-$ -Glc-Glc-Glc)	771, 609, 485
16	20.3	quercetin-feruloyl-pentaglucoside	340	1287	1111([M-H]$^-$ -Fer), 963([M-H]$^-$ -Hex-Hex), 787([M-H]$^-$ -Hex-Hex-Fer)	
17	21.2	kaempferol-sinapoyl-pentahexoside	330	1301	1139([M-H]$^-$ -Glc), 977([M-H]$^-$ -Glc-Glc), 771([M-H]$^-$ -Glc-Glc-Sin), 429	
18	21.7	kaempferol-sinapoyl-tetraglucoside	335	1139	977([M-H]$^-$ -Glc),785, 488	816, 771([M-H]$^-$ -Glc-Sin), 285([M-H]$^-$ -Glc-Sin-Glc-Glc-Glc)
19	21.9	kaempferol-tetrahexoside	335	933	609([M-H]$^-$ -Hex-Hex), 285[K]([M-H]$^-$ -Hex-Hex-Hex-Hex)	
20	22.4	kaempferol-feruloyl-pantahexoside	335	1271	1109 ([M-H]$^-$ -Hex), 947([M-H]$^-$ -Hex-Hex), 771([M-H]$^-$ -Hex-Hex-Fer), 609([M-H]$^-$ -Hex-Hex-Hex-Fer)	
21	22.8	kaempferol-feruloyl-tetrahexoside	330	1109	947([M-H]$^-$ -Glc), 827, 785([M-H]$^-$ -Glc-Glc), 623([M-H]$^-$ -Glc-Glc-Glc)	
22	23.3	methylquercetin-tetrahexoside	350	963	801([M-H]$^-$ -Hex), 639([M-H]$^-$ -Hex-Hex), 315([M-H]$^-$ -Hex-Hex-Hex-Hex	369, 477([M-H]$^-$ - Hex-Hex-Hex), 315
23	23.8	kaempferol-coumaroyl-trihexoside	320	917	755([M-H]$^-$ -Hex), 609([M-H]$^-$ -Hex-Coum), 285[K] ([M-H]$^-$ -Hex-Coum-Hex-Hex)	
24	26.9	kaempferol-tetrahexoside	335	935	773([M-H]$^-$ -Hex), 663, 637, 447([M-H]$^-$ -Hex-Hex-Hex), 285[K]([M-H]$^-$ -Hex-Hex-Hex-Hex)	
25	27.4	quercetin-dihexoside	350	625	301[Q]([M-H]$^-$ -Hex-Hex)	
26	27.8	cyanidin-tetrahexoside	520	935*	773([M+H]$^+$ -Hex), 449([M+H]$^+$ -Hex-Hex-Hex), 287[Cyan] ([M+H]$^+$ -Hex-Hex-Hex-Hex)	
		cyanidin-coumaroyl-triglucoside	520	919*	757([M+H]$^+$ -Glc), 449([M+H]$^+$ -Glc-Glc-Coum), 287[Cyan]([M+H]$^+$ -Glc-Glc-Glc-Coum)	
27	28.6	cyanidin-feruloyl-triglucoside	530	949*	787([M+H]$^+$ -Glc), 449([M+H]$^+$ -Glc-Glc-Fer), 287[Cyan]([M+H]$^+$ -Glc-Glc-Fer-Glc)	
28	28.7	kaempferol conjugate	325	965	803([M-H]$^-$ -Hex), 693([M-H]$^-$ -272), 447([M-H]$^-$ -518), 285[K] ([M-H]$^-$ -680)	
29	30.1	quercetin conjugate	310	855	693([M-H]$^-$ -Hex), 459, 447	469, 229
30	31.3	cyanidin-coumaroyl-triglucoside	520	919*	757([M+H]$^+$ -Glc), 449([M+H]$^+$ -Glc-Glc-Coum), 287[Cyan] ([M+H]$^+$ -Glc-Glc-Coum-Glc)	
31	32.0	cyanidin-3-feruloyldiglucoside-5-glucoside	520	949*	787([M+H]$^+$ Glc), 449([M+H]$^+$ -Glc-Glc-Fer), 287[Cyan]([M+H]$^+$ -Glc-Glc-Fer-Glc)	611([M+H]$^+$ -Glc-Fer), 465([M+H]$^+$ -Glc-Glc-Glc), 287[Cyan]([M+H]$^+$-Glc-Glc-Fer-Glc)
		cyanidin-3-sinapoyldiglucoside-5-glucoside		979*	817([M+H]$^+$ -Glc), 449([M+H]$^+$ -Glc-Glc-Sin), 287[Cyan]([M+H]$^+$ -Glc-Glc-Sin-Glc)	
32	34.9	cyanidin-coumaroyl-tetraglucoside	520	1081*	919([M+H] -Glc)	757([M+H]$^+$ -Glc-Glc), 449([M+H]$^+$ -Glc-Glc-Glc-Cou,), 287[Cyan]([M+H]$^+$ -Glc-Glc-Glc-Coum-Glc)
33	35.6	cyanidin-3-coumaroyl-sinapoyl-diglucoside-5-glucoside	535	1125*	963([M+H]$^+$ -Glc), 449([M+H]$^+$ -Glc-Coum-Sin-Glc). 287 [Cyan]([M+H]$^+$ -Glc-Coum-Sin-Glc-Glc)	
34	36.3	cyanidin-3-feruloyl-sinapoyl-diglucoside-5-glucoside	535	1155*	993([M+H]$^+$ -Glc)	818([M+H]$^+$ Glc-Fer), 449([M+H]$^+$ -Glc-Fer-Sin-Glc), 287[Cyan]([M+H]$^+$ -Glc-Fer-Sin-Glc-Glc)
35	37.3	cyanidin-diferuloyl-triglucoside	535	1125*	963([M+H]$^+$ -Hex), 810, 698, 451	787([M+H]$^+$ -Hex-Fer), 287[Cyan]([M+H]$^+$ -Hex-Fer-Hex-Fer)
36	38.7	kaempferol-sirapoyl-feruloyl-pentahexoside	330	1477	1153([M-H]$^-$-Hex-Hex),977([M-H]$^-$-Hex-Hex-Fer),947([M-H]$^-$-Hex-Hex-Sin),771([M-H]$^-$-Hex-Hex-Fer-Sin)	609([M-H]$^-$-Hex-Hex-Fer-Sin-Hex),285[K]([M-H]$^-$-Hex-Hex-Fer-Sin-Hex-Hex-Hex)
37	45.2	1,2-disinapoyl-diglucoside	330	753	529, 247	
38	46.8	1-sinapoyl-2-feruloyl-diglucoside	330	725	301	
39	47.9	1,2-diferuloyl-diglucoside	330	693	499, 259	
40	52.3	1,2,2-trisinapoyldiglucoside	325	951	736, 529	
41	53.9	1,2'-disinapoyl-2-feruloyldiglucoside	325	931	706	

Q, quercetin; K, kaempferol; Sin, sinapoyl; Glc, glucosyl; Hex, hexosyl; Cyan, cyanidin; Coum., coumaroyl; Fer, feruloyl
[a] Peak numbers and retention times refer to HPLC traces in Figure 4.11. * positive ionisation molecular ion ($[M+H]^+$)

Table 4.10. Summary of phenolic compounds detected in an extract of broccoli by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	λmax	[M-H]⁻ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
1	10.1	3-caffeoylquinic acid	325	353	191, 179	
2	14.2	quercetin-caffeoyl-dihexoside	315	787	625 ([M-H]⁻ -Caf), 462([M-H]⁻ -Caf-Hex)	
3	14.5	kaempferol-caffeoyl-hexoside	320	609	447([M-H]⁻ -Glc), 285[K]([M-H]⁻ -Glc-Glc)	
4	16.3	methylquercetin-dihexoside	340	639	519, 315[MetQ]([M-H]⁻ -Hex-Hex)	
5	16.7	quercetin-hexahexoside	330	1273	1111([M-H]⁻ -Hex), 949([M-H]⁻ -Hex-Hex), 787([M-H]⁻ -Hex-Hex-Hex),	949, 787, 625
6	18.2	quercetin-diglucoside	325-340	625	463([M-H]⁻ -Glc), 301[Q]([M-H]⁻ -Glc)	
7	18.9	kaempferol-hexahexoside	335	1257	933([M-H]⁻ -Hex-Hex), 771([M-H]⁻ -Hex Hex-Hex), 609*([M-H]⁻ -Hex-Hex-Hex-Hex), 429*	
8	19.4	quercetin-sinapoyl-pentahexoside	335	1317	1111([M-H]⁻ -Sin), 933, 787([M-H]⁻ -Sin-Hex-Hex)	
9	19.8	quercetin-sinapoyl-tetrahexoside	340	1155	993([M-H]⁻ -Hex), 949([M-H]⁻ -Sin), 787([M-H]⁻ -Sin-Hex), 625([M-H]⁻ -Sin-Hex-Hex), 463 ([M-H]⁻ -Sin-Hex-Hex-Hex)	
10	20.2	quercetin-feruloyl-pentahexoside	345	1287	1111([M-H]⁻ -Fer), 963([M-H]⁻ -Hex-Hex), 787([M-H]⁻ -Fer-Hex-Hex), 625([M-H]⁻ -Fer-Hex-Hex-Hex), 459	
11	20.6	quercetin-feruloyl-tetrahexoside	330,	1125	963([M-H]⁻ -Hex), 949([M-H]⁻ -Fer), 787([M-H]⁻ -Hex-Fer), 625([M-H]⁻ -Hex-Fer-Hex), 463*([M-H]⁻ -Hex-Fer-Hex-Hex)	
		quercetin coumaroyl-pentahexoside	340	1257	1111([M-H]⁻ -Hex), 933([M-H]⁻ -Hex-Hex), 787([M-H]⁻ -Hex-Hex-Coum), 625([M-H]⁻ -Hex-Hex-Coum-Hex),463*([M-H]⁻ -Hex-Hex-Coum-Hex-Hex)	
12	21.3	kaempferol-sinapoyl-pentahexoside	350	1301	977([M-H]⁻ -Hex-Hex), 771([M-H]⁻ -Hex-Hex-Sin), 753, 609*([M-H]⁻ -Hex-Hex-Sin-Hex)	
13	22.0	kaempferol-3-sophoroside	345	609	447([M-H]⁻ -Glc), 285[K]([M-H]⁻ -Glc-Glc)	
		kaempferol-sinapoyl-tetrahexoside	345	1139	815([M-H]⁻ -Hex-Hex), 609([M-H]⁻ -Hex-Hex -Sin), 429	
14	22.5	kaempferol-feruloyl-pentahexoside	335	1271	947([M-H]⁻ -Hex-Hex), 771([M-H]⁻ -Hex-Hex-Fer), 637	
15	23.4	methylquercetin-dihexoside	340	639	477([M-H]⁻ -Hex),315[MetQ]([M-H]⁻ -Hex-Hex)	
16	27.4	quercetin-diglucoside	340	625	301[Q]([M-H]⁻ -Glc-Glc), 271	
17	27.9	kaempferol-diglucoside	330	609	447([M-H]⁻ -Glc), 285[K]([M-H]⁻ -Glc Glc)	
18	31.6	kaempferol-diglucoside	350	609	429, 285[K]([M-H]⁻ -Glc-Glc)	
19	34.3	quercetin-3-glucoside	360	463	301[Q]([M-H]⁻ -Glc)	
20	34.8	quercetin-sinapoyl-hexahexoside	340	1479	1317([M-H]⁻ -Hex), 1155([M-H]⁻ -Hex Hex), 993 ([M-H]⁻ -Hex-Hex-Hex), 787([M-H]⁻ -Hex-Hex-Hex Sin), 625*([M-H]⁻ -Hex-Hex-Hex-Sin-Hex)	
21	36.0	quercetin-sinapoyl-pentahexoside	340	1317	1155([M-H]⁻ -Hex), 993([M-H]⁻ -Hex-Hex), 933, 801, 787([M-H]⁻ -Hex-Hex-Sin),625*([M-H]⁻ -Hex-Hex-Sin-Hex), 463*([M-H]⁻ -Hex-Hex-Sin-Hex-Hex)	
22	36.5	kaempferol-sinapoyl-hexahexoside	330	1463	1139([M-H]⁻ -Hex-Hex), 977([M-H]⁻ -Hex-Hex-Hex), 771([M-H]⁻ -Hex-Hex-Hex-Sin), 609([M-H]⁻ Hex-Hex-Hex-Sin-Hex), 447*([M-H]⁻ -Hex-Hex-Hex-Sin-Hex-Hex)	
23	37.0	quercetin-disinapoyl-pentahexoside	340	1523	1317([M-H]⁻ -Sin), 1199([M-H]⁻ -Hex-Hex), 993([M-H]⁻ -Hex-Hex-Sin), 787([M-H]⁻ -Hex-Hex-Sin-Sin), 625([M-H] -Hex-Hex-Hex Sin-Sin-Hex)	
24	38.1	kaempferol-feruloyl-trihexoside	330	947	785([M-H]⁻ -Hex), 623([M-H]⁻ -Hex-Hex), 609([M-H]⁻ -Hex-Fer), 285[K]([M-H] -Hex-Hex-Fer-Hex)	
25	38.5	kaempferol-disinapoyl-pentahexoside	335	1507	1183([M-H]⁻ -Hex-Hex), 977([M-H]⁻ -Hex-Hex-Sin), 771([M-H]⁻ -Hex-Hex-Sin-Sin), 653([M-H]⁻ -Hex-Hex Sin-Hex-Hex)	
		quercetin-disinapoyl-tetrahexoside	330	1361	1199([M-H]⁻ -Hex), 1155 ([M-H]⁻ -Sin), 993([M-H]⁻ -Hex-Sin), 787([M-H]⁻ -Hex-Sin-Sin), 463*([M-H]⁻ -Hex-Sin-Sin-Hex-Hex)	
26	39.5	quercetin-sinapoyl-feruloyl-tetrahexoside	335	1331	1169([M-H]⁻ -Hex), 1155([M-H]⁻ -Fer), 993([M-H]⁻ -Hex-Fer), 963([M-H]⁻ -Hex-Sin), 787([M-H]⁻ -Hex-Sin-Fer), 463*([M-H]⁻ -Hex-Sin-Fer-Hex-Hex)	
27	39.8	kaempferol-sinapoyl-feruloyl-pentahexoside	330	1477	1153 ([M-H]⁻ -Hex-Hex), 977([M-H]⁻ -Hex-Hex-Fer), 947([M-H]⁻ -Hex-Hex-Sin), 771([M-H]⁻ -Hex-Hex-Hex-Sin-Fer), 609*([[M-H]⁻ -Hex-Hex-Hex-Sin-Fer -Hex), 429	
28	40.1	kaempferol-disinapoyl-tetrahexoside	330	1345	1183([M-H]⁻ -Hex), 977([M-H]⁻ -Hex-Sin), 771([M-H]⁻ -Hex-Hex-Sin-Sin),	
29	40.6	kaempferol-sinapoyl-feruloyl-pentahexoside	330	1477	1153 ([M-H]⁻ -Hex-Hex), 947([M-H]⁻ -Hex-Hex-Sin), 771([M-H]⁻ -Hex-Hex-Sin-Fer), 609*([M-H]⁻ -Hex Hex-Sin-Fer Hex)	
		quercetin-sinapoyl-feruloyl-tetrahexoside	330	1331	1169 ([M-H]⁻ -Hex), 1125([M-H]⁻ -Sin), 963([M-H]⁻ -Hex-Sin), 949([M-H]⁻ -Sin-Fer), 765, 463*([M-H]⁻ -Sin-Fer Hex-Hex-Hex)	
30	41.5	kaempferol feruloyl-sinapoyl-tetrahexoside	330	1315	1153 ([M-H]⁻ -Hex), 977([M-H]⁻ -Hex- Fer), 771([M-H]⁻ -Hex-Fer-Sin), 351	
		kaempferol-diferuloyl-pentahexoside	330	1447	1125 ([M-H]⁻ -Hex-Hex), 947([M-H]⁻ -Hex-Hex-Fer), 771([M-H]⁻ -Hex-Hex-Fer-Fer)	
31	42.2	kaempferol-feruloyl-sinapoyl-tetrahexoside	330	1315	1153 ([M-H]⁻ -Hex), 1093, 977([M-H]⁻ -Hex- Fer), 771([M-H]⁻ -Hex-Fer-Sin), 351	
32	46.3	1,2-disinapoyl-diglucoside	330	753	529, 289	
33	47.5	1-sinapoyl-2-feruloyl-diglucoside	330	735	259, 500, 259	
34	48.3	1,2-diferuloyl-diglucoside	330	693	499, 259	
35	54.0	1,2,2 trisinapoyl-diglucoside	325	959	735, 511, 289	
36	55.2	1,2'-disinapoyl-2-feruloyl-diglucoside	325	931	705, 529, 427	
37	56.7	1-sinapoyl-2,2-diferuloyl-diglucoside	325	899	705, 511, 427, 289	

Q, quercetin; K, kaempferol; Sin, sinapoyl; Glc, glucosyl; Hex, hexosyl; Cyan, cyanidin; Coum, coumaroyl; Fer, feruloyl, * ions obtained after ISF (in-source fragmentation)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.12.

Table 4.11. Summary of phenolic compounds detected in an extract of kiwi by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	Absorbance(nm)	$[M-H]^-$ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
1	8.4	caffeic acid hexose conjugate	290	341	179 [CafAc]([M-H]$^-$ -Hex)	135
2	12.3	caffeic acid hexose conjugate	290	341	179 [CafAc]([M-H]$^-$ -Hex)	135
3	13.3	methoxycinnamic acid hexose conjugate	280, 335	339	177 [OCH_3-CinAc]([M-H]$^-$ -Hex)	
4	16.1	caffeic acid hexose conjugate	315	341	179 [CafAc]([M-H]$^-$ -Hex)	135
5	18.9	B2 dimer	280	577	425, 407, 289 ([M-H]$^-$ -Cat)	
6	20.9	(-)-epicatechin	280	289	245, 205, 179	
7	23	quercetin-hexosyl-rhamnoside	325	609	463([M-H]$^-$-Rham),447([M-H]$^-$-Glc),301[Q]([M-H]$^-$-Glc-Rham)	
8	24	catechin-trimer	280	867	697, 741, 577 ([M-H]$^-$ -Cat)	
9	33.4	quercetin-3-rutinoside	345	609	301[Q]([M-H]$^-$ -Glc-Rham), 271	255, 179
10	38.4	kaempferol-3-rutinoside	320, 340	593	285 [K]([M-H]$^-$ -Glc-Rham)	
11	40	kaempferol-3-glucoside	325	447	285 [K]([M-H]$^-$ -Glc),327, 255	
12	40.4	quercetin rhamnoside	345	447	301[Q]([M-H]$^-$ -Rham)	179, 255, 151
13	41.8	hesperidin	285	609	301[Hesp]([M-H]$^-$ -Glc-Rham)	286, 242

CafAc, caffeic acid; Hex, Hexosyl; CinAc, cinnamic acid; Hesp, hesperitin; Cat, catechin; Glc, glucosyl; Rham, rhamnosyl;
[a] Peak numbers and retention times refer to HPLC traces in Figure 4.13

Table 4.12. Summary of phenolic compounds detected in an extract of clementine by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
1	8.9	caffeic acid-conjugate	245, 275	379	341([M-H]$^-$ -38) -179[CafAc] ([M-H]$^-$ -Hex-38)	
2	21.4	nobiletin	325	401	357, 277, 233	
3	22.6	3,3',4',5,6,7,8-heptamethoxyflavone	290, 325	431	401, 270	
4	26.8	naringenin-rutinoside-hexoside	280	741	433([M-H]$^-$ -Rut), 271[Narin]([M-H]$^-$ -Rut-Hex)	151-177
5	31.4	eriocitrin (eridictyol-3-rutinoside)	285	595	287[Erid]([M-H]$^-$ -Rut)	
6	33.3	naringenin conjugate	280	783	475([M-H]$^-$- Rham-Hex)	271[Narin], 429, 415, 433
7	33.3	quercetin-3-rutinoside	320	609	301 [Q]([M-H]$^-$- Rut), 343, 179	271, 255, 179
8	37.8	narirutin (naringenin-3-rutinoside)	280	579	271 [Narin]([M-H]$^-$ -Rut)	151, 177
9	41.7	hesperidin (hesperitin-3-rutinoside)	280, 325	609	301[Hesp]([M-H] -Rut), 343, 325	286, 242
10	45.2	hesperitin-conjugate	280, 325	763	671, 301[Hesp]	286, 242, 125
11	54.7	neoponcirin (isosakuranetin-rutinoside)	280, 330	593	285([M-H]$^-$ -Rut)	243, 164

CafAc, caffeic acid; Hex, hexosyl; Narin, naringenin; Rut, rutinosyl; Erid, eridictyol; Hesp, hesperitin; Glc, glucosyl

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.14

Table 4.13. Summary of phenolic compounds detected in an extract of green pepper by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
1	12.6	caffeic acid hexose conjugate	285	341	179[CafAc]([M-H]$^-$- Hex)	
2	16.4	caffeic acid hexose conjugate	285	341	179[CafAc]([M-H]$^-$- Hex)	
	17.5	5-caffeoylquinic acid	325	353	191	
3	22.8	quercetin-rhamnosyl-hexoside	345	609	463([M-H]$^-$ -Rham), 447([M-H]$^-$-Hex), 301[Q]([M-H]$^-$ -Rham-Hex)	
4	34.7	quercetin-3-glucoside	330	463	301[Q]([M-H]$^-$- Glc), 179	
5	35.2	luteolin-pentose-hexoside	345	579	447([M-H]$^-$- Pent), 285[Lut]([M-H]$^-$- Hex-Pent)	
6	40.2	quercetin-rhamnoside	350	447, 301	271,255,179	
7	41.6	hesperidin	280	609	301[Hesp]([M-H]$^-$-Rham-Glc)	286,242
8	42.4	luteolin-hexose-pentose-malonate	345	621	489([M-H]$^-$-Pent), 285[Lut] ([M-H]$^-$ -Pent-204)	

Table 4.14. Summary of phenolic compounds detected in an extract of yellow pepper by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
3	22.8	quercetin-pentose-dihexoside	345	771	609([M-H]$^-$-Hex), 463([M-H]$^-$ -Hex-Pent), 301[Q] ([M-H]$^-$-Hex-Pent-Hex)	
		quercetin-rhamnosyl-hexoside	345	609	463([M-H]$^-$ -Rham), 447([M-H]$^-$-Hex), 301[Q]([M-H]$^-$ -Rham-Hex)	
7	416	hesperidin	280	609	301[Hesp]([M-H]$^-$-Rham-Glc)	286,242
8	42.4	luteolin-hexose-pentose-malonate	345	621	489([M-H]$^-$-Pent), 285[Lut] ([M-H]$^-$ -Pent-204)	

Table 4.15. Summary of phenolic compounds detected in an extract of red pepper by HPLC with diode array and MS^2 detection[a]

Peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
3	22.7	quercetin-pentose-dihexoside	345	771	609([M-H]$^-$-Hex), 463([M-H]$^-$ -Hex-Pent), 301[Q] ([M-H]$^-$-Hex-Pent-Hex)	271(ISF)
		quercetin-rhamnosyl-hexoside	345	609	463([M-H]$^-$ -Rham), 447([M-H]$^-$-Hex), 301[Q]([M-H]$^-$ -Rham-Hex)	
6	40.1	quercetin-rhamnoside	340	447	301([M-H]$^-$-Rham)	
7	416	hesperidin	280	609	301([M-H]$^-$-Rham-Glc)	242
8	42.4	luteolin-hexose-pentose-malonate	345	621	489([M-H]$^-$-Pent), 285[Lut] ([M-H]$^-$ -Pent-204)	

CafAc, caffeic acid Q, quercetin; Lut, luteolin; Hesp, hesperitin; Hex, hexosyl; Rham, rhamnosyl; Glc, glucosyl; Pent, pentosyl; ISF, in source fragmentation

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.15, 4.16 and 4.17

Table 4.16. Summary of phenolic compounds detected in an extract of cherry tomato by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
1	10.8	caffeic acid hexose conjugate	290	341	179[CafAc]([M-H]- Hex)	135
2	15.2	caffeic acid hexose conjugate	290	341	179[CafAc]([M-H]- Hex)	
3	16.6	3-caffeoylquinic acid	325	353	191, 179, 173, 135	
4	19.4	quercetin-3-rutinoside-7- glucoside	340	771	609([M-H]-Hex)-301[Q]([M-H]-Hex-Rut)	
5	21.1	5-caffeoylquinic acid	320	353	191, 179	
6	30.7	quercetin-rutinoside-pentoside	355	741	609([M-H]-Pent),-301[Q]([M-H]-Pent-Rut)	271, 255, 217
7	33.3	quercetin-3-rutinoside	355	609	301[Q]([M-H]-Glc- Rham)	271, 255, 179
8	34.6	kaempferol-rutinoside-pentoside	325	725	593([M-H]- -Pent), 284[K]([M-H]-Glc-Pent-Rham)	
9	38.4	kaempferol-3-rutinoside	330	593	285[K]([M-H]-Glc- Rham)	255-191
10	43.2	4,5-dicaffeoylquinic acid	325	515	353 ([M-H]-Caf), 173	
11	57.5	tricaffeoylquinic acid	330	677	515 ([M-H]- Caf)	353([M-H]- -Caf-Caf), 335, 179
12	61.9	naringenin	280	271	151, 177	

CafAc, caffeic acid; Hex, hexosyl; Q, quercetin; Pent, pentosyl; Rham, rhamnosyl; Caf, caffeoyl; Rut, rutinosyl

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.18

Table 4.17. Summary of phenolic compounds detected in an extract of plums by HPLC with diode array and MS^3 detection[a]

Peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$(m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
1	13.0	procyanidin dimer	280	577	425, 289 ([M-H]$^-$-Cat)	245
2	13.9	procyanidin polymer	245-280	1439	1151([M-H]$^-$-Cat), 863([M-H]$^-$ -Cat-Cat), 575([M-H]$^-$ -Cat-Cat-Cat), 1989, 1672	
3	15.2	(+)-catechin	280	289	245, 205, 179	
4	15.8	procyanidin dimer	245-280	575	287([M-H]$^-$-Cat), 413, 219	
5	16.4	procyanidin pentamer	245-280	1441	1271, 1153([M-H]$^-$-Cat), 865([M-H]$^-$ -Cat-Cat),575 ([M-H]$^-$ -Cat-Cat-Cat)	
6	16.9	procyanidin trimer	280	865	577([M-H]$^-$-Cat), 695	
		cyanidin-3-glucoside	515	449*	287 [Cyan]([M+H]$^+$-Glc)	
7	17.7	procyanidin dimer	245-280	577	425, 289 ([M-H]$^-$-Cat)	
8	18.4	procyanidin polymer	280	865	695, 577, ([M-H]$^-$Cat) 1309	
		cyanidin-3-rutinoside	515	595*	449([M+H]$^+$-Rham), 287[Cyan]([M+H]$^+$-Rham-Glc)	
9	19.1	procyanidin dimer	245-280	577	425, 289 ([M-H]$^-$-Cat)	
10	20.4	procyanidin hexamer	280	1729	1441, ([M-H]$^-$-Cat), 1153([M-H]$^-$-Cat -Cat), 863([M-H]$^-$-Cat -Cat-Cat), 575([M-H]$^-$-Cat -Cat-Cat-Cat)	
11	21.2	(-)-epicatechin	280	289	245, 205, 179	
12	22.2	procyanidin polymer	280	865	575, 1605, 1441, 1152	
13	23.2	procyanidin polymer	280	1441	1315, 1151([M-H]$^-$-Cat), 865([M-H]$^-$-Cat-Cat), 576([M-H]$^-$-Cat-Cat-Cat), 1847, 1710	
14	24.1	procyanidin dimer	280	577	425, 289 ([M-H]$^-$-Cat)	
15	24.7	procyanidin polymer	245-280	1441	1151([M-H]$^-$-Cat), 865([M-H]$^-$-Cat-Cat), 575([M-H]$^-$-Cat-Cat-Cat), 1891	
16	25.2	procyanidin polymer	280	1729	1440([M-H]$^-$-Cat), 1151 ([M-H]$^-$-Cat-Cat), 863([M-H]$^-$-Cat-Cat-Cat), 1603, 1891	
17	25.9	procyanidin polymer	280	1441	1151([M-H]$^-$-Cat), 863([M-H]$^-$-Cat-Cat), 575([M-H]$^-$-Cat-Cat-Cat), 1889	
18	26.7	procyanidin polymer	245-280	867	741, 579([M-H]$^-$-Cat), 497, 287([M-H]$^-$-Cat-Cat), 1114	
19	27.4	procyanidin hexamer	245-280	1730	1441([M-H]$^-$-Cat), 1153([M-H]$^-$-Cat -Cat), 863([M-H]$^-$-Cat -Cat-Cat), 575([M-H]$^-$-Cat -Cat-Cat-Cat)	863, 711, 491, 1313
20	28.6	procyanidin polymer	245-280	865	739, 695, 577([M-H]$^-$-Cat), 289([M-H]$^-$-Cat-Cat), 1151, 1439	
21	29.8	procyanidin hexamer	245-280	1729	1441, ([M-H]$^-$-Cat), 1153([M-H]$^-$-Cat -Cat), 863([M-H]$^-$-Cat -Cat-Cat), 575([M-H]$^-$-Cat -Cat-Cat-Cat)	
22	32.9	quercetin-3-rutinoside	355	609	301[Q] ([M-H]$^-$ -Glc-Rham)	271, 255, 179
23	33.3	procyanidin pentamer	280	1441	1271, 1153([M-H]$^-$-Cat), 865([M-H]$^-$ -Cat-Cat)	
24	33.7	procyanidin dimer	280	577	425, 289 ([M-H]$^-$-Cat)	
25	34.4	quercetin-3-glucoside	355	463	301[Q]([M-H]$^-$ -Glc),-	271, 255, 179
26	36.6	quercetin-pentose	355	433	301[Q]([M-H]$^-$ -Pent),-	271, 255, 179
27	36.9	quercetin-pentosyl-pentoside	350	565	415, 301[Q]([M-H]$^-$ -Pent-Pent)	271, 255
28	39.8	quercetin-pentose	350	433	301[Q]([M-H]$^-$-Pent)	271, 255, 179
29	40.2	quercetin-3-rhamnoside	350	447	301[Q]([M-H]$^-$ -Rham)	271, 255, 179
30	41.6	quercetin-malonate-hexoside	355	505	463(M$^-$-(Mal-COOH), 301[Q]([M-H]$^-$ -Mal-Hex]	255, 179, 151

Cyan, cyanidin. Q, quercetin: Cat, catechin, Mal, malonyl; Pent, pentosyl; Hex, hexosyl. glcUA, glucuronyl; Glc, glucosyl; Rham, rhamnosyl
[a] Peak numbers and retention times refer to HPLC traces in Figure 4.19 * positive ionisation molecular ion ([M+H]$^+$)

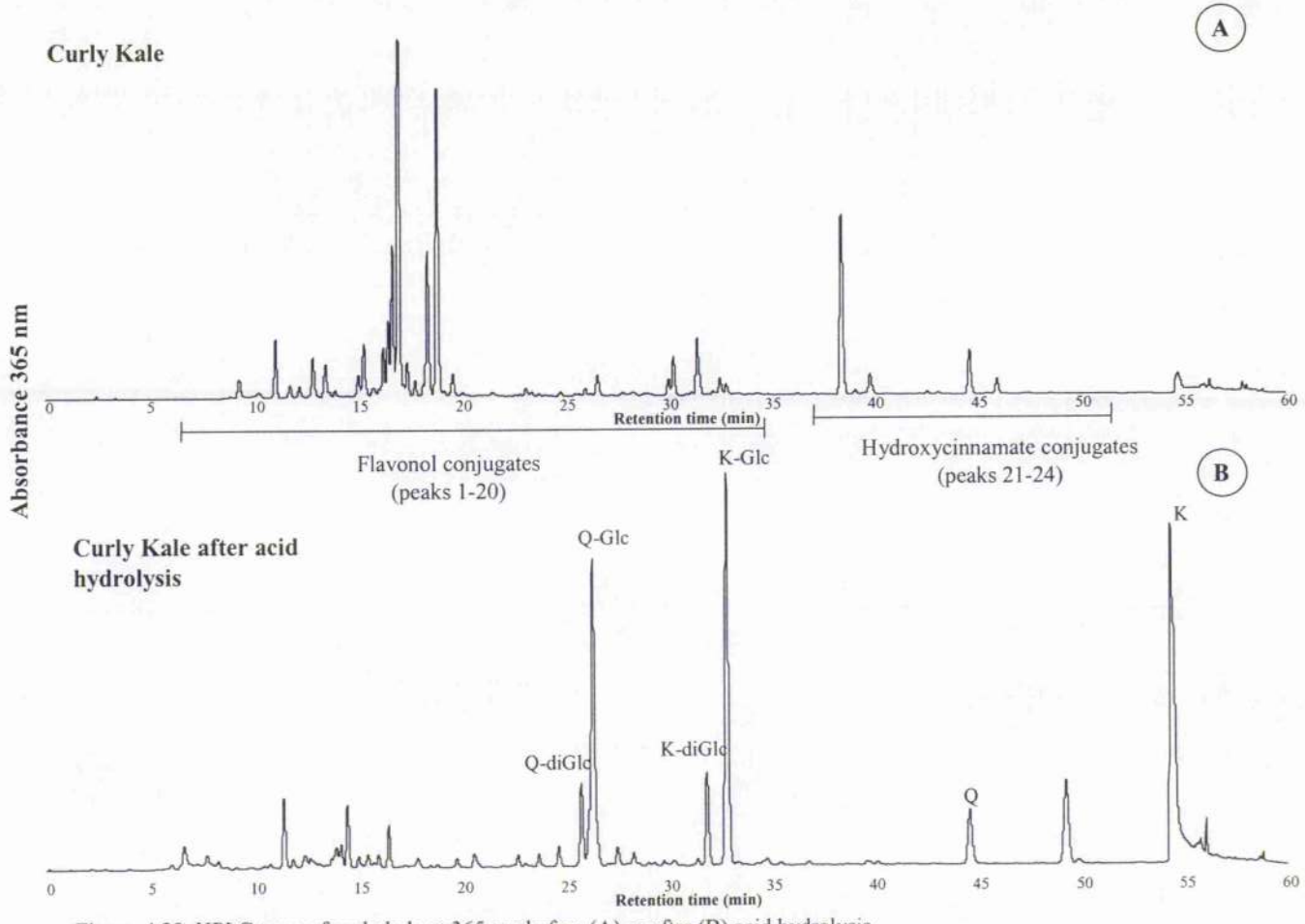


Figure 4.20. HPLC trace of curly kale at 365 nm before (A) or after (B) acid hydrolysis
Q, quercetin, Glc, glucoside; K, kaempferol

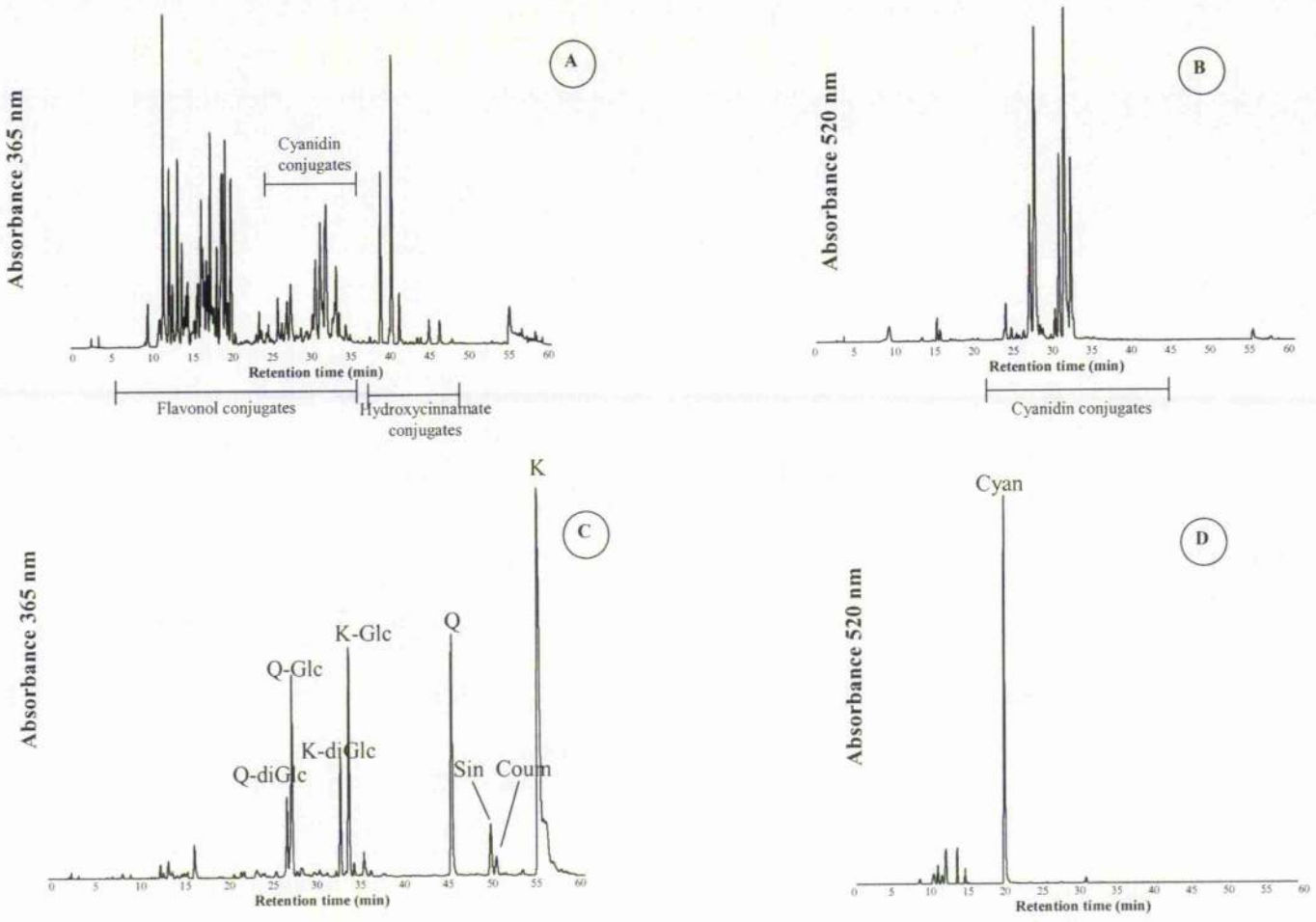


Figure 4.21.. HPLC trace of purple broccoli at 365 nm (A) before or after (C) acid hydrolysis and 520 nm before (B) or after (D) acid hydrolysis

Q, quercetin, Glc, glucoside; K, kaempferol, Cyan, cyanidin; Sin, sinapoyl residue; Coum, coumaroyl residue

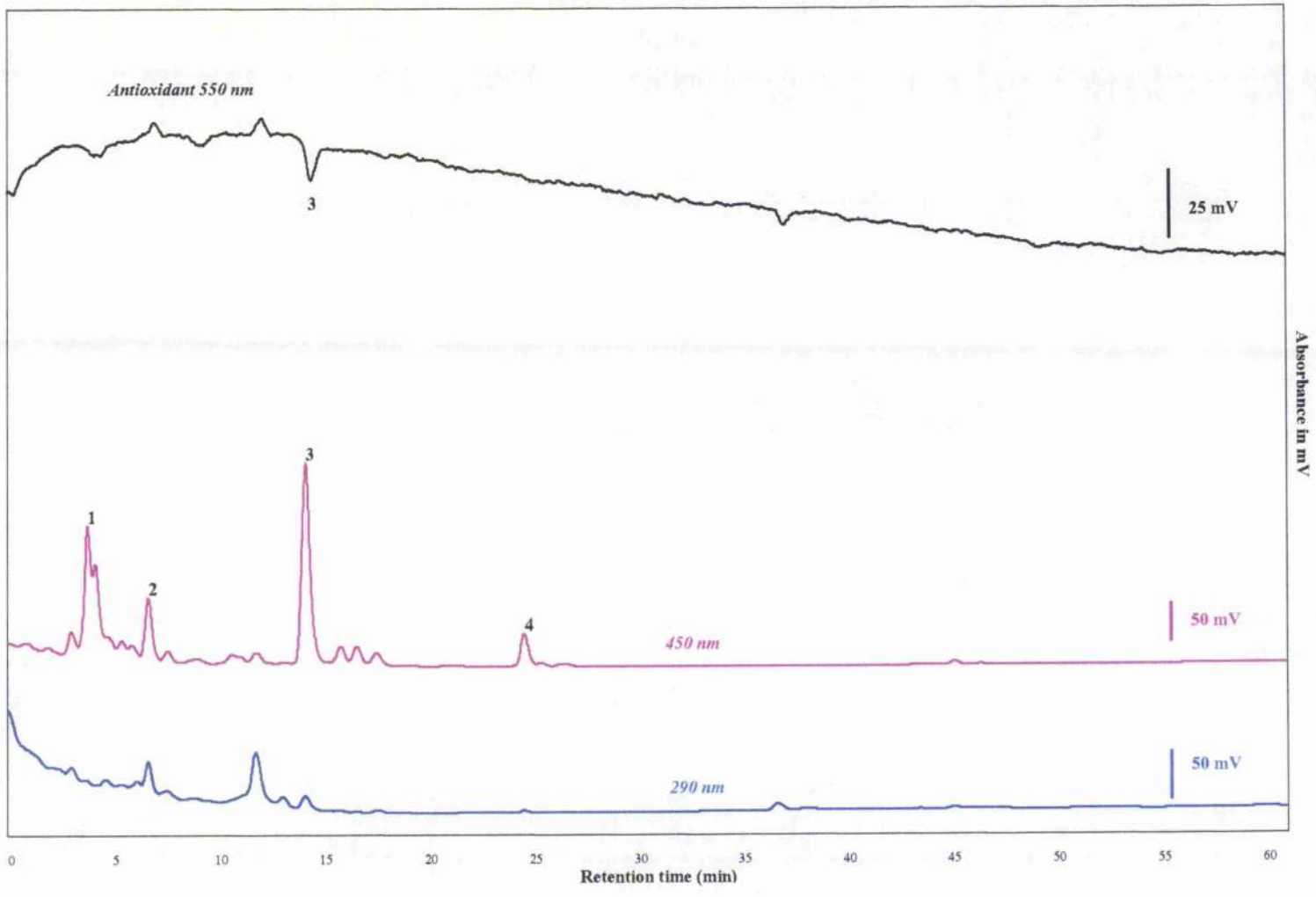


Figure 4.22 HPLC-carotenoid trace for Curly Kale

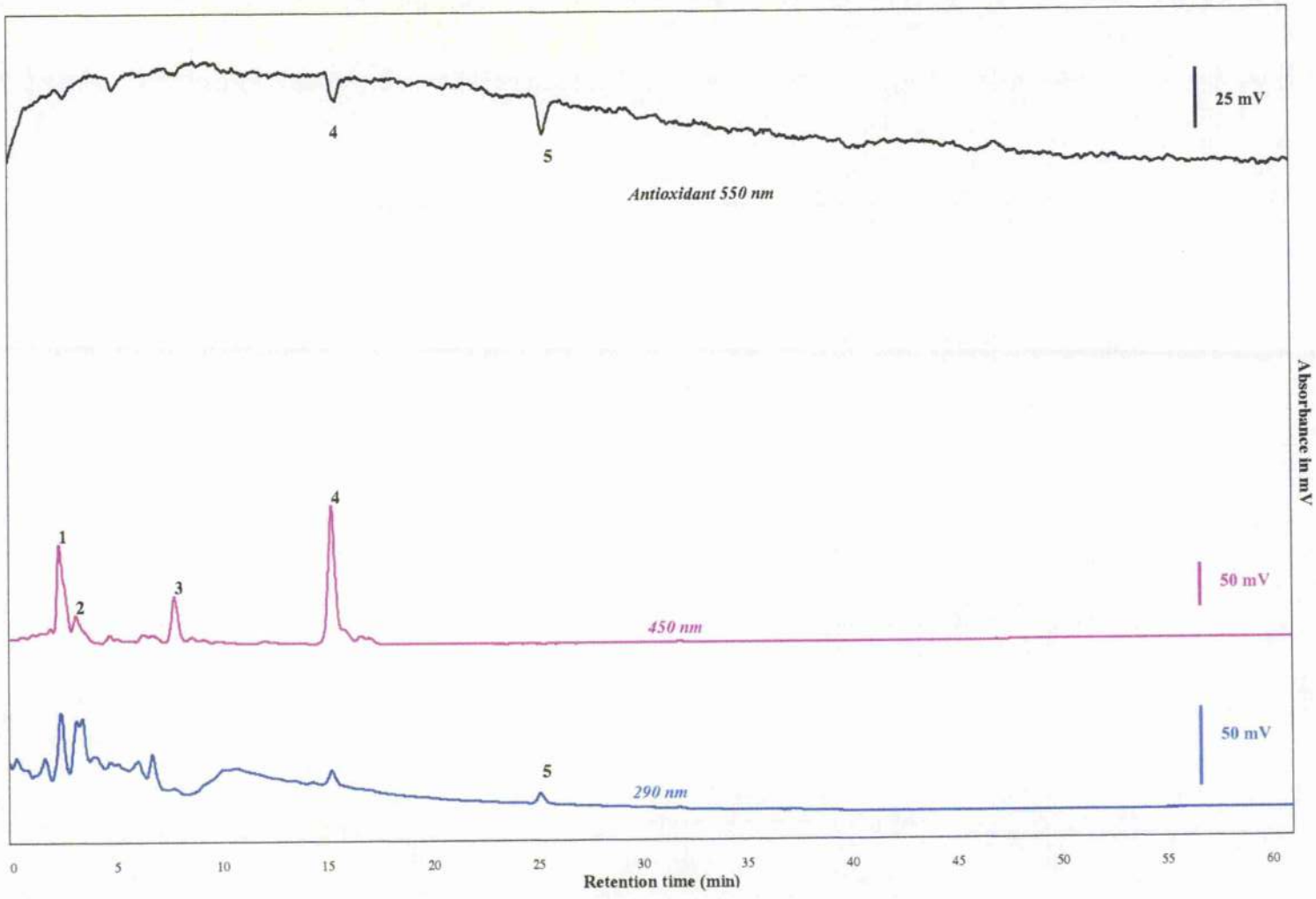


Figure 4.23 HPLC-carotenoid trace for Green Pepper

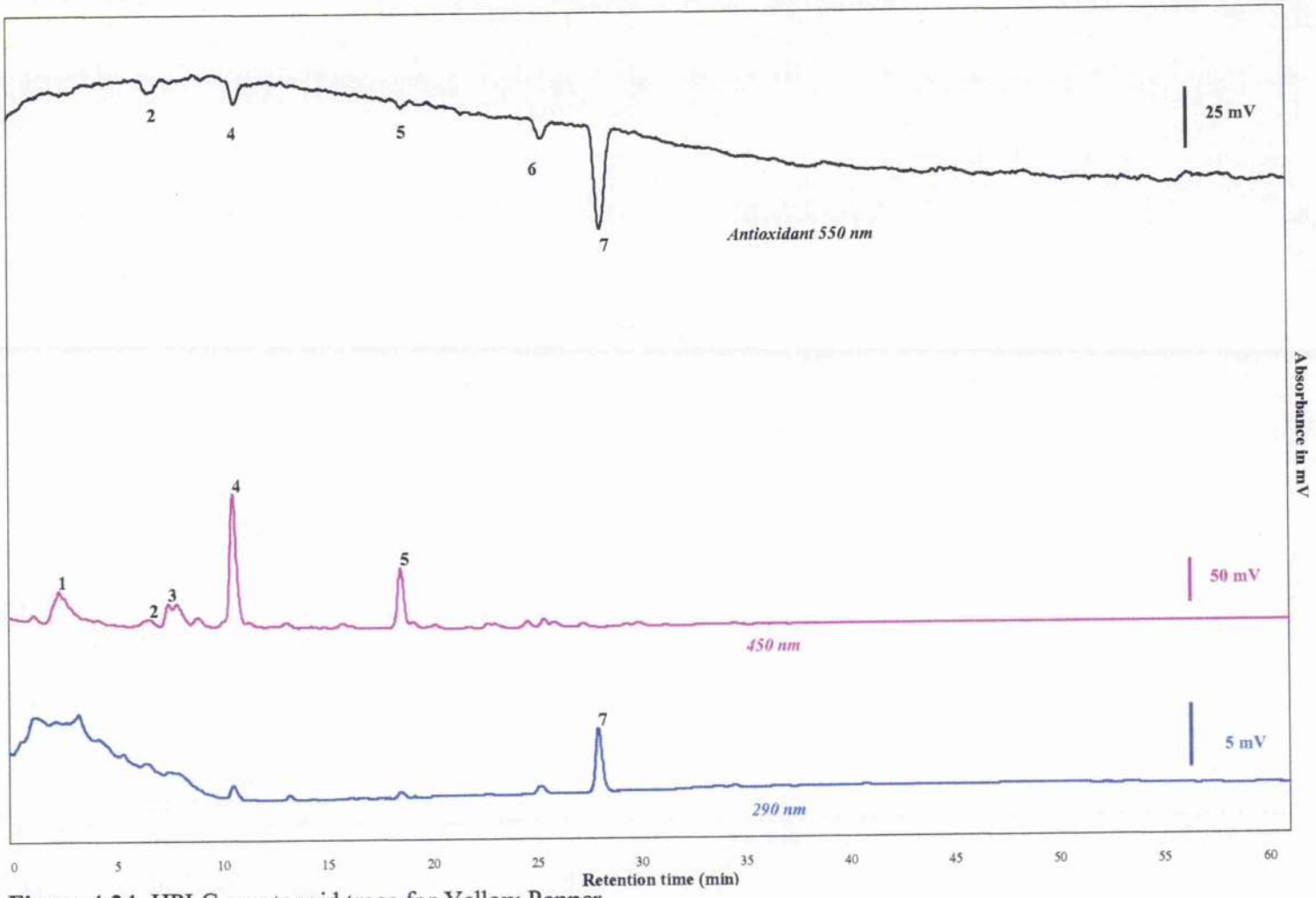


Figure 4.24. HPLC-carotenoid trace for Yellow Pepper

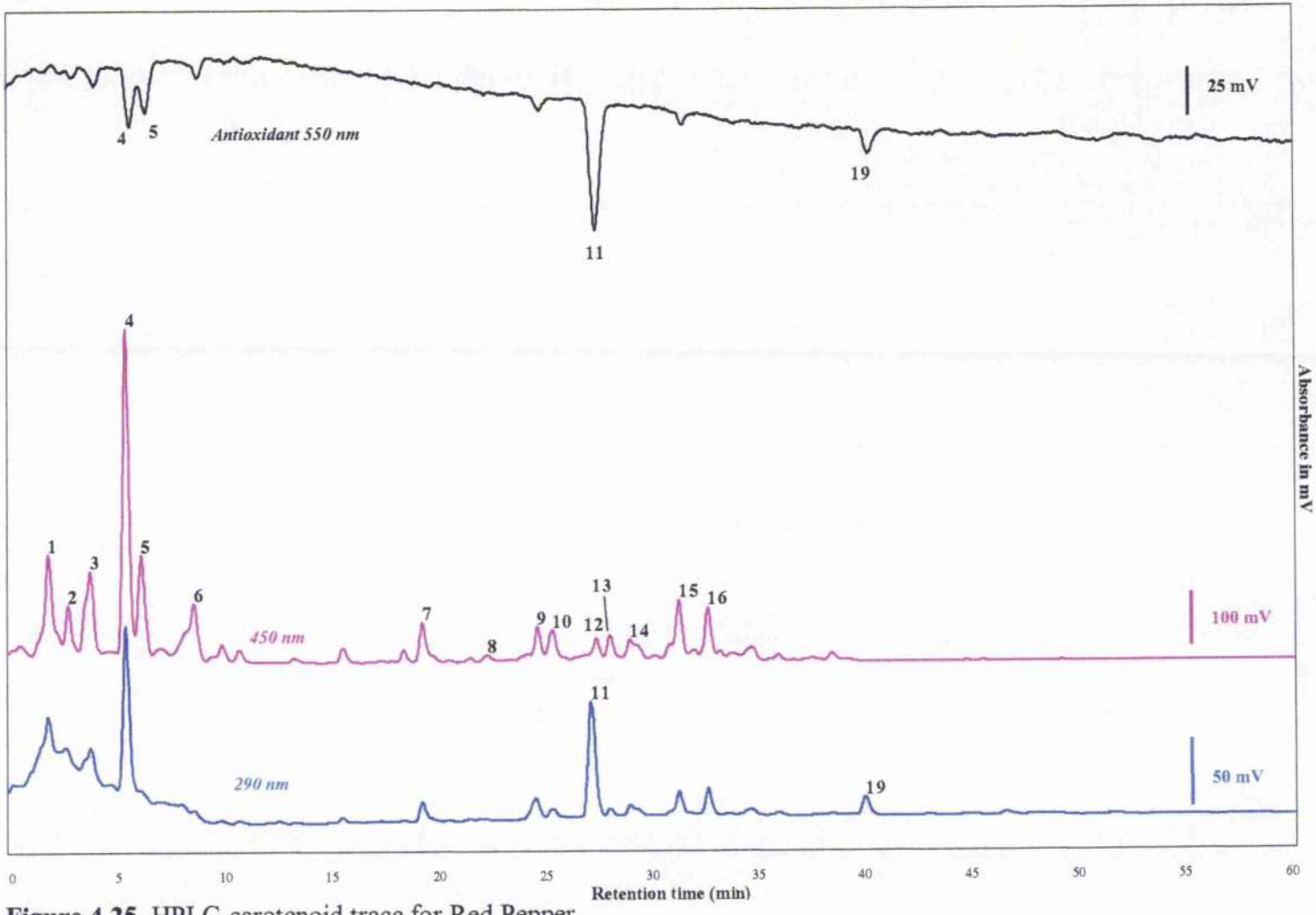


Figure 4.25. HPLC-carotenoid trace for Red Pepper

Table 4.18. Antioxidant activity and levels of individual phenolic compounds in raspberries.

			Quantity		Antioxidant activity	
Peak	Compound	t_R	µg/g	nmoles/g	Trolox equiv. (nmoles/g)	Percentage
Vitamin C		3.2	178.4 ± 5.3	1013.6 ± 30.1	680.6 ± 5.4	7.4
Phenolic compounds						
1	cyanidin-3-sophoroside	22.1	190.8 ± 9.8	425.9 ± 21.9	484.9 ± 22.4	5.3
2	cyanidin-3-(2^G-glucosylsophoroside) cyanidin-3-sambubioside cyanidin-3-galactoside	24.2	130.7 ± 3.7	291.6 ± 8.3	362.4 ± 19.2	4.0
3	pelargonidin-3-sophoroside	25.5	11.3 ± 0.5	25.3 ± 1.0	25.3 ± 4.3	0.3
4	cyanidin-3-rutinoside cyanidin-3-xylosylrutinoside (-)epicatechin	26.9	28.5 ± 0.9 n.q.	63.6 ± 1.9 n.q.	253.0 ± 6.9	2.8
5	pelargonidin-3-glucoside pelargonidin-3(2^Gglucosylrutinoside) sanguiin H-10	27.9	7.2 ± 0.2 7.6 ± 0.2	16.1 ± 0.5 17.0 ± 0.4	161.5 ± 70.28	1.8
6	lambertianin C	34.7	404.6 ± 3.0	2379.9 ± 17.8	2579.8 ± 13.8	28.1
7	sanguiin H-6	36.2	576.0 ± 3.4	3388.0 ± 19.7	3788.2 ± 17.4	41.3
8	ellagic acid pentose	38.9	3.6 ± 0.1	12.0 ± 0.2	n.d.	0.0
9	ellagic acid	40.3	5.1 ± 0.1	16.8 ± 0.4	n.d.	0.0
10	quercetin-galactosylrhamnoside	42.9	1.3 ± 0.1	2.7 ± 0.2	n.d.	0.0
11	quercetin-3-rutinoside	44.0	1.4 ± 0.0	3.0 ± 0.0	n.d.	0.0
12	quercetin-3-galactoside	44.4	3.3 ± 0.1	7.1 ± 0.2	n.d.	0.0
13	quercetin-3-glucuronide	45.4	3.4 ± 0.1	7.4 ± 0.1	n.d.	0.0
14	methyl-ellagic acid-pentose	49.9	1.4 ± 0.1	4.7 ± 0.3	n.d.	0.0
15	ellagic acid-4-acetylxyloside	50.1	1.2 ± 0.0	3.8 ± 0.2	n.d.	0.0
16	ellagic acid-4-acetylarabinoside	52.1	1.5 ± 0.1	4.9 ± 0.6	n.d.	0.0
					Total polyphenol	**83.5**
					TOTAL HPLC	**90.9**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.3 Also see Table 4.1. n.q. – not quantified; n.d. – not detected

Table 4.19. Antioxidant activity and levels of individual phenolic compounds in blueberries.

			Quantity		Antioxidant activity	
Peak	Compound	t_R	µg/g	nmol/g	Trolox equiv.(nmol/g)	Percentage
Vitamin C		3.2	20.2 ± 0.8	114.8 ± 4.5	n.d	0
Phenolic compounds						
1	delphinidin-3-galactoside	17.8	461.5 ± 4.3	1030.2 ± 9.7	1526.5 ± 76.6	15.2
2	5-caffeoylquinic acid	21.0	390.2 ± 3.9	870.9 ± 8.7	1213.4 ± 53.6	12.1
3	cyanidin-3-glucoside delphinidin-3-arabinoside	21.3	104.0 ± 2.5	754.0 ± 18.3	1144.9 ± 53.4	11.4
4	petunidine-3-galactoside	23.0	244.7 ± 2.6	546.2 ± 5.7	914.6 ± 90.8	9.1
5	cyanidin-3-arabinoside	24.2	45.5 ± 0.8	101.6 ± 1.9	124.8 ± 12.0	1.2
6	petunidin-3-arabinoside peonidin-3-galactoside	26.3	169.1 ± 1.8	377.5 ± 4.1	569.0 ± 51.8	5.7
7	malvidine-3-galactoside	27.8	454.4 ± 4.39	1014.4 ± 9.7	1280.2 ± 89.1	12.8
8	malvidine-3-glucoside delphinidin-6-acetyl-3-glucoside	28.6	85.9± 0.9	191.8 ± 2.0	268.2 ± 13.3	2.7
9	peonidin-3-arabinoside	29.2	21.3 ± 0.4	47.7 ± 0.8	n.d	0.0
10	malvidine-3-arabinoside	31.0	373.8 ± 3.8	834.3 ± 8.6	1231.7± 64.9	12.3
11	myricetin-3-galactoside	32.7	39.2 ± 0.6	124.2 ± 1.8	168.5 ± 3.9	1.7
12	petunidin-6-acetyl-3-glucoside	33.4	34.6 ± 0.4	77.2 ± 0.9	76.1 ± 11.2	0.8
13	myricetin-pentose / 5-feruoylquinic acid	35.5	14.8 ± 0.2 n.q	46.8 ± 0.8 n.q	269.3 ± 3.7	2.7
14	malvidin-6-acetyl-3-glucoside	38.1	155.1± 1.9	346.2 ± 4.2	442.3 ± 32.2	4.4
15	quercetin-3-rutinoside / quercetin-3-galactoside	39.3	163.4± 2.4	352.1 ± 5.1	229.8 ± 16.2	2.3
16	quercetin-3-glucoside/ unknown flavonol	40.1	95.3 ± 1.3	205.4 ± 2.75	153.4 ± 10.0	1.5
17	quercetin-3-arabinoside	43.4	40.9 ± 0.5	88.1 ± 1.1	n.d	0.0
					Total polyphenol	**95.8**
					TOTAL HPLC	**95.8**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.4 Also see Table 4.2 n.q. – not quantified; n.d. – not detected

Table 4.20.. Antioxidant activity and levels of individual phenolic compounds in blackcurrants

Peak	Compound	t_R	Quantity µg/g	Quantity nmol/g	Antioxidant activity Trolox equiv.(nmol/g)	Antioxidant activity Percentage
Vitamin C		3.2	508.0 ± 17.5	2886.4 ± 99.4	1046.5 ± 37	**10.7**
Phenolic compounds						
1	caffeic-acid glucoside conjugate	14.9	5.4 ± 0.2	39.1 ± 1.7	45.6 ± 4.1	0.5
2	delphinidin-3-galactoside	17.9	283.4 ± 11.9	632.6 ± 26.6	768.4 ± 91.5	7.8
3	delphinidin-3-glucoside	19.5	460.2 ± 19.3	1027.3 ± 43.1	1304.2 ± 88.1	13.3
4	delphinidin-3-rutinoside/ cyanidin-3-galactoside/ delphinidin-3-arabinoside/ 5-caffeoylquinic acid	21.7	884.0 ± 36.9 8.4 ± 0.5	1973.2 ± 82.5 46.8 ± 3.0	2211.9 ± 125.0	22.6
5	cyanidin-3-glucoside	23.4	278.8 ± 11.5	622.3 ± 25.6	797.8 ± 57.4	8.1
6	petunidin-3-galactoside	24.1	89.5 ± 4.1	199.9 ± 9.0	265.5 ± 5.0	2.7
7	cyanidin-3-arabinoside cyanidin-3-rutinoside petunidin-3-glucoside	25.7	676.0± 28.4	1509.0 ± 63.5	1688.6 ± 82.6	17.2
8	peonidin-3-galactoside petunidin-3-rutinoside	27.9	85.3 ± 4.1	190.4 ± 9.1	193.6 ± 6.2	2.0
9	malvidin-3-galactoside peonidin-3-glucoside	29.8	147.3 ± 6.8	328.7 ± 15.2	305.4 ± 14.7	3.1
10	malvidin-3-glucoside peonidin-pentose peonidin-3-rutinoside	31.6	175.8± 8.1	392.4 ± 18.2	278.2 ± 79.4	2.8
11	malvidin-3-arabinoside	33.5	46.1 ± 2.7	102.9 ± 6.1	94.9± 24.0	1.0
12	myricetin-3-rutinoside	35.4	24.9 ± 1.2	78.4 ± 3.7	131.8 ± 19.9	1.3
13	myricetin-glucuronide	36.1	25.8± 1.1	81.1 ± 3.6	77.6 ± 26.1	0.8
14	myricetin-3-malonylglucoside	42.7	2.9 ± 0.1	9.2 ± 0.3	n.d	0.0
15	quercetin-3-rutinoside	43.2	23.7 ± 0.9	51.1 ± 2.0	37.9 ± 1.2	0.4
16	quercetin-3-galactoside	43.9	20.6 ± 0.8	44.3 ± 1.8	16.3± 4.1	0.2
17	quercetin-3-glucuronide/quercetin-3-glucoside	44.0	43.5 ± 1.9	93.7 ± 4.1	54.0 ± 6.9	0.6
18	delphinidin-3-*O*-6''-coumaroylglucoside	44.9	8.9 ± 0.4	19.9 ± 0.9	n.d	0.0
19	quercetin-3-malonylglucoside	48.1	7.4 ± 0.4	15.9 ± 0.8	n.d	0.0
20	kaempferol-3-rutinoside	48.8	6.6 ± 0.2	14.7 ± 0.5	n.d	0.0
21	kaempferol-3-glucoside	50.7	5.3 ± 0.2	11.7 ± 0.5	n.d	0.0
22	unknown	52.3	10.2 ± 0.4	22.7 ± 0.9	n.d	0.0
					Total polyphenol	**84.5**
					TOTAL HPLC	**95.3%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.5 Also see Table 4.3 n.q. – not quantified; n.d. – not detected

Table 4.21. Antioxidant activity and levels of individual phenolic compounds in cranberries

Peak	Compound	t_R	Quantity		Antioxidant activity	
			µg/g	nmol/g	Trolox equiv.(nmol/g)	Percentage
Vitamin C		3.2	194.9 ± 0.5	1107.4 ± 2.8	437.3 ± 19	10.5
Phenolic compounds						
1	procyanidin dimer	16.7	123.8 ± 0.4	426.9 ± 1.4	30.4 ± 1.0	0.73
2	(−)-catechin	18.9	56.1 ± 0.5	193.3 ± 1.8	61.9 ± 4.6	1.48
3	cyanidin-3-galactoside	21.2	69.7± 1.3	155.6 ± 2.9	234.1 ± 4.9	5.61
	p-coumaric acid hexose conjugate		33.2±1.2	184.3± 6.5		
4	5-caffeoylquinic acid	21.7	8.9 ± 0.2	64.3 ± 1.4	73.3 ± 1.7	1.75
5	procyanidin dimer	23.4	108.4 ± 9.0	373.7 ± 31.1	188.8 ± 3.7	4.52
6	cyanidin-3-arabinose	24.4	72.5 ± 0.5	161.9 ± 1.1	264.4 ± 8.9	6.33
	procyanidin hexamer		691.5 ± 24.2	2384.5 ± 83.5		
7	(-)epicatechin	25.4	149.3 ± 16.3	514.7 ± 56.3	366.6 ± 5.2	8.78
8	peonidin-3-galactoside	26.4	124.5± 0.6	277.9 ± 1.4	324.5 ± 21.2	7.77
9	malvidin-3-glucoside	28.5	11.1 ± 0.1	24.9 ± 0.3	115.3 ± 13.6	2.76
	procyanidin polymer		31.5 ± 1.1	108.6 ± 3.8		
10	peonidin-3-arabinoside	29.7	78.4 ± 0.4	175.1 ± 0.9	232.9 ± 10.1	5.58
11	malvidin-3-arabinoside	31.2	4.4 ± 0.0	9.8 ± 0.1	11.0 ± 5.6	0.26
12	myricetin-3-galactoside	33.0	43.2 ± 0.1	135.9 ± 0.4	243.0 ± 6.2	5.82
	procyanidin dimer		117.3 ± 12.7	404.4 ± 43.8		
13	myricetin-3-arabinoside	37.5	17.2± 0.0	54.0 ± 0.1	89.8± 4.2	2.15
	procyanidin polymer		35.3 ± 9.1	121.8 ± 31.4		
14	procyanidin dimer	38.2	68.8± 6.0	237.2± 20.7		
15	quercetin-3-galactoside	39.4	137.9 ± 0.6	297.3 ± 1.4	671.4 ± 20.9	16.08
	procyanidin trimer		297.4 ± 19.1	1025.6 ± 65.7		
16	quercetin-3-xylopyranoside	42.9	15.9 ± 0.2	34.2 ± 0.5	39.2 ± 3.6	0.94
17	quercetin-3-arabinopyranoside	43.6	12.7 ± 0.2	27.4 ± 0.4	64.2 ± 1.7	1.49
18	quercetin-3-arabinofuranoside	44.1	43.6 ± 0.2	94.0 ± 0.4		
19	quercetin-3-rhamnoside	46.5	27.7± 0.1	59.8 ± 0.3	36.6 ± 5.2	0.88
					Total phenolics	**73.0**
					TOTAL HPLC	**81.9%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.6 Also see Table 4.4 n.q. – not quantified; n.d. – not detected

Table 4.22.. Antioxidant activity and levels of individual phenolic compounds in blackberries.

			Quantity		Antioxidant activity	
Peak	Compound	t_R	µg/g	nmol/g	Trolox equiv.(nmol/g)	Percentage
Vitamin C		3.2	69.1 ± 1.3	392.6 ± 7.4	479.0 ± 1.2	4.0
Phenolic compounds						
1	cyanidin-3-glucoside	22.5	1335.6 ± 10.2	2981.3 ± 22.9	3440.3 ± 180.2	28.5
2	cyanidin-3-rutinoside	24.4	102.8 ± 0.4	229.4 ± 1.0	349.2 ± 29.5	2.9
3	sanguiin H-10	25.2	30.6 ± 0.2	161.1 ± 1.3	307.4 ± 5.1	2.5
4	sanguiin	29.2	95.8 ± 0.8	504.2 ± 4.3	479.0± 3.9	4.0
5	lambertianin C	30.4	347.8 ± 1.9	1830.5 ± 10.1	2412.2 ± 36.9	20.0
6	cyanidin-malonylglucoside	30.8	46.0 ± 0.5	102.7 ± 1.2		
7	sanguiin H-6	32.1	175.7 ± 0.9	924.7 ± 5.0	1729.1 ± 20.8	14.3
8	cyanidin-rutinoside	33.0	72.7 ± 0.8	162.2 ± 1.9	246.1 ± 32.0	2.0
	ellagic acid-pentose conjugate	n.q	n.q	n.q		
9	unknown ellagic acid conjugate	37.5	5.4 ± 0.0	11.7 ± 0.1	n.d	0.0
10	quercetin-rutinoside	37.5	7.1 ± 0.0	15.3 ± 0.1	n.d	0.0
11	quercetin-3-galactoside	39.1	11.1 ± 0.1	23.9 ± 0.3	n.d	0.0
12	quercetin-3-glucuronide/ quercetin-3-glucoside	39.9	47.3 ± 0.2	101.8 ± 0.5	45.0 ± 6.7	0.4
13	quercetin-malonate-hexoside	43.3	18.5 ± 0.2	39.8 ± 0.4	n.d	0.0
14	kaempferol-glucuronide	45.8	6.8 ± 0.1	15.1 ± 0.2	n.d	0.0
					Total phenolics	74.7
					TOTAL HPLC	78.7

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.7 Also see Table 4.5 n.q. – not quantified; n.d. – not detected

Table 4.23.. The antioxidant activity and levels of individual phenolic compounds in strawberries.

			Quantity		Antioxidant activity	
Peak	Compound	t_R	µg/g	nmol/g	Trolox equiv.(nmol/g)	Percentage
Vitamin C		3.2	397.3 ± 14.5	2257.4 ± 82.4	675.9 ± 5.5	**24.3**
Phenolic compounds						
1	procyanidinin dimer	16.3	27.2 ± 2.6	93.9 ± 9.1	50.1 ± 2.4	1.8
2	procyanidinin dimer	17.1	51.0 ± 1.7	175.9 ± 6.0	105.1 ± 3.2	3.8
3	procyanidinin trimer	18.5	77.5 ± 6.1	267.1 ± 21.1	181.4 ± 20.5	6.5
4	*p*-coumaric hexose	20.5	5.5 ± 0.2	39.7 ± 1.3	n.d	0.0
5	pelargonidin-3-glucoside	25.4	191.5 ± 3.3	427.4 ± 7.5	401.5 ± 31.7	14.5
6	pelargonidin-3-*O*-(6-*O*-malonyl-glucoside)	33.7	61.8 ± 0.9	137.9 ± 2.1	175.3 ± 24.4	6.3
7	ellagic acid rhamnoside	36.2	6.0 ± 0.2	12.8 + 0.5	n.d	0.0
8	sanguiin	37.8	74.6 ± 4.6	438.6 ± 27.3	594.7 ± 16.2	21.4
9	quercetin-3-glucuronide	39.5	4.8 ± 0.3	10.4 ± 0.6	n.d	0.0
10	kaempferol-3-glucoside	44.9	3.1 ± 0.3	6.9 ± 0.6	n.d	0.0
11	kaempferol-hexoside-malonate	49.3	15.8 ± 0.4	35.3 + 0.8	n.d	0.0
					Total polyphenols	**54.3**
					TOTAL HPLC	**78.6**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.8 Also see Table 4.6 n.q. – not quantified; n.d. – not detected

Table 4.24.. The antioxidant activity and levels of individual phenolic compounds in redcurrants.

			Quantity		Antioxidant activity	
Peak	Compound	t_R	µg/g	nmol/g	Trolox equiv.(nmol/g)	Percentage
Vitamin C		3.3	174.0 ± 7.2	988.6 ± 40.7	816.1 ± 19.2	**56.4**
Phenolic compounds						
1	4-hydroxybenzoic acid glucoside	11.2	18.5± 0.4	102.5 ± 2.0	n.d	0.0
2	caffeic acid hexose conjugate	13.9	4.8± 0.0	26.7 ± 0.2	27.7± 2.4	1.9
3	caffeic acid hexose conjugate	14.8	1.4± 0.1	7.9 ± 0.5	n.d	0.0
4	cyanidin-3-sambubioside	21.9	28.6± 0.7	63.8 ± 1.7	56.4 ± 4.0	3.9
5	cyanidin-3-rutinoside, cyanidin 3-xylosyl-rutinoside	22.8	169.9± 4.1	379.2 ± 9.2	264.5 ± 19.9	18.3
6	myricetin-rutinoside	32.0	0.7± 0.0	2.3 ± 0.1	n.d	0.0
7	myricetin-rhamnoside	37.4	2.3± 0.1	7.2 ± 0.2	28.9 ± 3.2	2.0
8	quercetin-3-rutinoside	37.9	12.9 ± 0.3	27.8 ± 0.7	18.2 ± 3.6	1.3
9	quercetin-3-glucoside	39.4	5.0± 0.1	10.7± 0.3	n.d	0.0
10	quercetin-hexoside-malonate	43.2	2.9± 0.1	6.5 ± 0.2	n.d	0.0
11	kaempferol-rutinoside	44.9	1.5± 0.0	3.3± 0.1		
12	kaempferol-3-glucoside	45.3	2.8± 0.1	6.0 ± 0.1		
					Total Polyphenol	**26.3**
					TOTAL HPLC	**82.7%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.9 Also see Table 4.7 n.q. – not quantified; n.d. – not detected

Table 4.25. Antioxidant activity and levels of individual phenolic compounds in curly kale.

Peak	Compound	Quantity		Antioxidant activity	
		µg/g	nmol/g	Trolox equiv.(nmol/g)	Percentage
Vitamin C		246.8 ± 1.6	1402 ± 8.9	327.2 ± 13.5	**18.7**
Phenolic compounds					
1	quercetin-triglucoside / 3-caffeoylquinic acid	19.8 ± 0.7	42.7* ± 1.5	189.4 ± 16.1	10.8*
2	quercetin-3-diglucoside-7-glucoside	27.3 ± 1.5	58.9 ± 3.2	15.3 ± 2.8	0.9
3	kaempferol conjugate (dihexoside+ unidentified 198 *amu* group)	8.9 ± 0.9	19.8 ± 2.1	n.d.	0.0
4	kaempferol-3-diglucoside-7-glucoside	32.7 ± 1.8	72.9 ± 4.1	n.d.	0.0
5	quercetin conjugate (trihexoside+ unidentified 192 *amu* group)	21.7 ± 1.0	46.8 ± 2.2	30.7 ± 1.9	1.8
6	kaempferol conjugate (dihexoside+ unidentified 192 *amu* group)	23.0 ± 0.9	51.3 ± 2.1	32.9 ± 1.0	1.9
7	quercetin-3-feruloyldiglucoside-7-glucoside	40.5 ± 1.1	87.4 ± 2.4	55.4 ± 1.4	3.2
8	quercetin-tetraglucoside	24.1 ± 0.4	52.0 ± 0.9	12.1 ± 1.2	0.7
9	quercetin-sinapoyl-tetraglucoside	45.3 ± 0.6	97.6 ± 1.2	n.d.	0.0
10	kaempferol conjugate(sinapoyltrihexoside+unidentified 198 *amu* group)	104.1 ± 1.3	232.3 ± 3.0	32.9 ± 9.3	1.9
11	quercetin-3-sinapoyldiglucoside-7-glucoside	305.7 ± 1.5	658.9 ± 3.3	117.4 ± 29.6	6.7
12	quercetin-feruoyl-triglucoside	30.3 ± 0.9	65.3 ± 1.9	n.d.	0.0
13	kaempferol-3-sinapoyltriglucoside-7-glucoside	126.5 ± 1.7	282.4 ± 3.8	40.6 ± 7.6	2.3
14	kaempferol-sinapoyl-triglucoside	276.6 ± 2.0	617.3 ± 4.5	45.8 ± 10.2	2.6
15	kaempferol-sinapoyl-tetraglucoside	9.3 ± 0.4	20.8 ± 0.9	n.d.	0.0
16	quercetin-3-sinapoyldiglucoside	19.7 ± 0.7	42.4 ± 1.4	n.d.	0.0
17	quercetin-3-rutinoside	4.7 ± 0.4	10.2 ± 0.8	n.d.	0.0
18	quercetin-3-disinapoyltriglucoside-7-diglucoside	22.6 ± 0.7	48.7 ± 1.6	30.3 ± 2.9	1.7
19	quercetin-disinapoyl-tetraglucoside	43.0 ± 0.7	92.7 ± 1.5	60.1 ± 1.4	3.4
20	kaempferol-3-disinapoyltriglucoside-7-glucoside	12.1 ± 0.5	27.0 ± 1.1	10.1 ± 0.2	0.6
21	1,2-disinapoylglucoside	23.3 ± 0.2	129.4 ± 1.1	214.2 ± 26.8	12.3
22	1-sinapoyl-2-feruloyldiglucoside	7.9 ± 0.1	43.8 ± 0.5	16.3 ± 2.4	0.9
23	1,2,2'-trisinapoyldiglucoside	8.3 ± 0.1	46.3 ± 0.5	87.5 ± 0.4	5.0
24	1,2'-disinapoyl-2-feruloyldiglucoside	5.1 ± 0.0	28.5 ± 0.2	30.2 ± 2.0	1.7
Carotenoids and tocopherols				**Total phenolics**	58.4
1	unknown carotenoid (UV 450nm)	40.0 ± 1.2	74.5 ± 2.1	n.d.	0.0
2	unknown carotenoid (UV 450nm)	18.8 ± 4.0	35.1 ± 7.4	n.d.	0.0
3	lutein	40.7 ± 1.8	75.9 ± 3.4	19.2 ± 2.8	1.1
4	unknown carotenoid (UV 450-470nm)	5.3 ± 0.0	10.0 ± 0.1	n.d.	0.0
5	α-tocopherol	3.8 ± 0.2	8.9 ± 0.5	5.0 ± 0.2	0.3
6	α-carotene	1.2 ± 0.3	2.2 ± 0.6	n.d.	0.0
7	β-carotene	0.3 ± 0.1	0.5 ± 0.1	n.d.	0.0
				Total carotenoids	1.4
				TOTAL HPLC	**78.5%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.10 Also see Table 4.8 n.q. – not quantified; n.d. – not detected

Table 4.26. Antioxidant activity and levels of individual phenolic compounds in purple broccoli.

		Quantity		Antioxidant activity	
Peak	**Compound**	**µg/g**	**nmol/g**	**Trolox equiv.(nmol/g)**	**Percentage**
Vitamin C				981.1 ± 30.6	24.7
Phenolic compounds					
1	cyanidin-3-diglucoside-5-glucoside	4.1 ± 0.0	9.2 ± 0.0		
2	3-caffeoylquinic acid	10.4 ± 0.6	58.0 ± 3.3	293.8 ± 26.8	7.2
3	quercetin-trihexoside	4.1 ± 0.1	8.9 ± 0.3	n.d.	
4	quercetin-tetrahexoside	60.9 ± 0.1	131.3 ± 0.2	79.8 ± 7.5	2.0
5	quercetin-tetrahexoside	39.5 ± 0.9	85.1 ± 2.0	26.0 ± 6.3	0.6
6	kaempferol conjugate (dihexoside+unidentified 198 *amu* group)	17.7 ± 0.1	39.5 ± 0.2	n.d.	
7	kampferol-sinapoyl-diglucoside	40.7 ± 0.9	90.9 ± 2.1	n.d.	
8	kaempferol conjugate (trihexoside+unidentified 198 *amu* group)	19.8 ± 2.7	44.2 ± 8.2	n.d.	
9	quercetin-pentahexoside	2.3 ± 0.0	5.0 ± 0.1	28.8 ± 9.7	0.7
10	quercetin-hexahexoside	10.2 ± 2.0	21.9 ± 4.4	30.9 ± 3.6	0.8
11	kaempferol conjugate (pentahexoside+unidentified 192 *amu* group)	33.3 ± 1.8	74.5 ± 4.1	n.d.	
12	kaempferol conjugate (tetrahexoside+unidentified 192 *amu* group)	23.5 ± 1.8	52.4 ± 4.1	64.5 ± 12.9	1.6
13	quercetin-trihexoside	20.2 ± 1.5	43.5 ± 3.3	30.7 ± 12.8	0.8
14	quercetin-tetrahexoside	46.5 ± 2.2	100.2 ± 4.7	40.6 ± 9.4	1.0
15	kaempferol-pentahexoside	24.1 ± 1.4	53.9 ± 3.2	35.0 ± 9.2	0.9
16	quercetin-feruloyl-pentaglucoside	61.1 ± 2.0	131.6 ± 4.3	45.7 ± 4.9	1.1
17	kaempferol-pentahexoside	37.6 ± 1.8	84.0 ± 4.0	n.d.	
18	kaempferol-sinapoyl-tetraglucoside	129.1 ± 3.4	288.2 ± 7.5	27.4 ± 0.3	0.7
19	kaempferol-tetrahexoside				
20	kaempferol-feruloyl-pentahexoside	83.2 ± 1.8	185.7 ± 4.0	26.1 ± 3.3	0.6
21	kaempferol-feruloyl-tetrahexoside	18.0 ± 1.2	40.1 ± 2.7	n.d.	
22	methylquercetin-tetrahexoside	58.8 ± 1.6	126.8 ± 3.5	n.d.	
23	kaempferol-coumaroyl-trihexoside	6.1 ± 1.4	13.7 ± 3.1	n.d.	
24	kaempferol-tetrahexoside	2.4 ± 0.7	5.5 ± 1.7	n.d.	
25	quercetin-dihexoside	15.4 ± 0.7	33.1 ± 1.5	23.8 ± 1.3	0.6
26	cyanidin-tetrahexoside cyanidin-coumaroyl-triglucoside	10.2 ± 0.1	22.9 ± 0.3	36.2 ± 1.2	0.9
27	cyanidin-feruloyl-triglucoside	3.1 ± 0.1	6.9 ± 0.2	22.5 ± 1.3	0.6
28	kaempferol conjugate	9.0 ± 1.1	20.0 ± 2.5	n.d.	
29	quercetin conjugate	24.0 ± 1.2	53.6 ± 2.6	24.3 ± 1.1	0.6
30	cyanidin-coumaroyl-triglucoside	42.2 ± 0.2	94.3 ± 0.4	94.0 ± 10.4	2.3
31	cyanidin-3-feruloyldiglucoside-5-glucoside/ cyanidin-3-sinapoyldiglucoside-5-glucoside	90.2 ± 0.4	201.3 ± 0.8	248.0 ± 11.9	6.1
32	cyanidin-coumaroyl-tetraglucoside	8.1 ± 0.1	18.0 ± 0.2	39.6 ± 7.4	1.0
33	cyanidin-3-coumaroyl-sinapoyl diglucoside-5-glucoside	43.2 ± 0.2	96.4 ± 0.4	170.5 ± 5.9	4.2
34	cyanidin-3-feruloyl-sinapoyl-diglucoside-5-glucoside	107.1 ± 0.4	239.0 ± 1.0	292.2 ± 5.1	7.2
35	cyanidin-diferuloyl-triglucoside	48.8 ± 1.7	109.0 ± 3.9	102.5 ± 4.2	2.5
36	kaempferol-sinapoyl-feruloyl-pentahexoside	51.5 ± 3.0	111.1 ± 6.4	33.3 ± 4.0	0.8
37	1,2-disinapoyl-diglucoside	10.6 ± 0.0	59.2 ± 0.1	114.8 ± 0.3	2.8
38	1-sinapoyl-2-feruloyldiglucoside	33.0 ± 0.7	183.2 ± 3.8	137.8 ± 3.2	3.4
39	1,2-diferuloyldiglucoside	10.7 ± 0.0	59.5 ± 0.2	n.d.	
40	1,2, 2-trisinapoyldiglucoside	3.0 ± 0.1	16.6 ± 0.7	22.4 ± 1.1	0.6
41	1,2'-disinapoyl-2-feruloyldiglucoside	3.5 ± 0.1	19.7 ± 0.8	16.9 ± 1.1	0.4

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.11 Also see Table 4.9 n.q. - not quantified; n.d. – not detected

Table 4.27. Antioxidant activity and levels of individual phenolic compounds in broccoli.

		Quantity		Antioxidant activity	
Peak	**Compound**	**μg/g**	**nmol/g**	**Trolox equiv.(*nmol/g*)**	**Percentage**
Vitamin C					
Phenolic compounds					
1	3-caffeoylquinic acid	29.4 ± 1.9	166.2 ± 10.7	197.4 ± 19.0	17.8
2	quercetin-caffeoyl-dihexoside	13.5 ± 1.1	29.1 ± 2.4	n.q.	0
3	kaempferol-caffeoyl-hexoside	22.0 ± 1.3	49.0 ± 2.9	n.q.	0
4	methylquercetin-dihexoside	14.3 ± 1.3	30.8 ± 2.9	29.3 ± 4.3	2.6
5	quercetin-hexahexoside	25.4 ± 1.9	54.7 ± 4.1	10.1 ± 2.9	0.9
6	quercetin-diglucoside	16.5 ± 1.2	35.5 ± 2.5	10.3 ± 1.8	0.9
7	kaempferol-hexahexoside	35.5 ± 4.1	79.3 ± 9.1	22.4 ± 2.3	2.0
8	quercetin sinapoyl-pentahexoside	6.7 ± 1.1	14.3 ± 2.4	n.q.	0
9	quercetin-sinapoyl-tetrahexoside	11.8 ± 1.1	25.5 ± 2.3	5.4 ± 0.4	0.5
10	quercetin-feruloyl-pentahexoside	14.5 ± 0.3	31.2 ± 0.6	4.9 ± 0.3	0.4
11	quercetin-feruloyl-tetrahexoside/ quercetin coumaroyl-pentahexoside	26.9 ± 1.5	58.0 ± 3.3	11.9 ± 2.3	1.1
12	kaempferol-sinapoyl-pentahexoside	10.9 ± 1.1	24.4 ± 2.5	n.q.	0
13	kaempferol-3-sophoroside/ kaempferol-sinapoyl-tetrahexoside	30.8 ± 2.3	68.8 ± 5.1	68.5 ± 3.2	6.2
14	kaempferol-feruloyl-pentahexoside	17.8 ± 1.4	39.8 ± 3.2	n.q.	0
15	methylquercetin-dihexoside	29.9 ± 2.4	64.5 ± 5.2	n.q.	0
16	quercetin-3-diglucoside	6.5 ± 0.5	14.0 ± 1.1	7.4 ± 0.6	0.7
17	kaempferol-diglucoside	3.6 ± 0.1	8.1 ± 0.1	n.q	0
18	kaempferol-diglucoside	16.7 ± 0.8	37.2 ± 1.9	n.q.	0
19	quercetin-3-glucoside	5.1 ± 0.1	11.0 ± 0.1	4.4 ± 0.4	0.4
20	quercetin-sinapoyl-hexahexoside	7.1 ± 0.2	15.3 ± 0.4	10.8 ± 0.8	1.0
21	quercetin-sinapoyl-pentahexoside	7.8 ± 0.2	16.9 ± 0.4	9.1 ± 0.4	0.8
22	kaempferol-sinapoyl-hexahexoside	21.7 ± 0.8	48.5 ± 1.8	17.3 ± 1.7	1.6
23	quercetin-disinapoyl-pentahexoside	11.6 ± 0.6	25.0 ± 1.3	10.5 ± 1.3	0.9
24	kaempferol-feruloyl-trihexoside	24.6 ± 2.3	55.0 ± 5.2	21.9 ± 1.6	2.0
25	kaempferol-disinapoyl-pentahexoside/ quercetin-disinapoyl-tetrahexoside	45.9 ± 1.7	102.6 ± 3.7	41.5 ± 1.5	3.7
26	quercetin-sinapoyl-feruloyl-tetrahexoside	16.9 ± 0.6	36.3 ± 1.4	19.6 ± 0.2	1.8
27	kaempferol-sinapoyl-feruloyl-pentahexoside	30.1 ± 1.2	67.2 ± 2.8	16.2 ± 1.2	1.5
28	kaempferol-disinapoyl-tetrahexoside	30.1 ± 1.2	67.2 ± 2.7	20.2 ± 0.3	1.8
29	kaempferol-sinapoyl-feruloyl-pentahexoside/ quercetin-sinapoyl-feruloyl-tetrahexoside	7.5 ± 3.2	16.8 ± 7.1	8.2 ± 1.4	0.7
30	kaempferol-feruloyl-sinapoyl-tetrahexoside/ kaempferol-diferuloyl-pentahexoside	24.5 ± 0.8	54.7 ± 1.8	10.8 ± 0.9	1.0
31	kaempferol-feruloyl-sinapoyl-tetrahexoside	9.3 ± 0.0	20.7 ± 0.1	4.1 ± 0.4	0.4
32	1,2-disinapoyl-diglucoside	44.6 ± 1.9	247.7 ± 10.8	56.0 ± 3.9	5.1
33	1-sinapoyl-2-feruloyl-diglucoside	26.8 ± 1.1	148.8 ± 6.3	93.0 ± 9.2	8.4
34	1,2-diferuloyl-diglucoside	7.6 ± 0.3	42.1 ± 1.9	n.q.	0
35	1,2,2-trisinapoyl-diglucoside	7.8 ± 0.4	43.5 ± 2.1	49.4 ± 3.5	4.5
36	1,2'-disinapoyl-3-feruloyl-diglucoside	11.3 ± 0.4	62.8 ± 2.1	66.5 ± 3.1	6.0
37	1-sinapoyl-2,2-diferulnyl-diglucoside	4.2 ± 0.2	23.5 ± 1.3	24.5 ± 15.6	2.2
				Total phenolics	**76.9%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.12 Also see Table 4.10 n.q. – not quantified; n.d. – not detected

Table 4.28. Antioxidant activity and levels of individual phenolic compounds in kiwi.

Peak	Compound	Quantity		Antioxidant activity	
		µg/g	nmol/g	Trolox equiv.(nmol/g)	Percentage
vitamin C		584.9 ± 15.9	3323.2 ± 90.2	693.8 ± 15.2	68.7
Phenolic compounds					
1	caffeic acid-hexose conjugate	2.4 ± 0.1	13.3 ± 0.5	n.d.	0.0
2	caffeic acid-hexose conjugate	3.3 ± 0.3	18.4 ± 1.5	n.d.	0.0
3	methoxycinnamic acid hexose conjugate	4.0 ± 0.4	22.3 ± 2.0	n.d.	0.0
4	caffeic acid-hexose conjugate	13.9 ± 1.0	77.3 ± 5.5	n.d.	0.0
5	dimer B2	6.2 ± 0.4	21.4 ± 1.2	13.1 ± 9.5	1.3
6	(-)-epicatechin	14.9 ± 0.5	51.4 ± 1.6	30.7 ± 23.3	3.9
7	quercetin-hexosyl-rhamnoside	0.3 ± 0.0	0.6 ± 0.0	n.d.	0.0
8	catechin trimer	7.3 ± 0.2	25.0 ± 0.6	n.d.	0.0
9	quercetin-3- rutinoside	1.0 ± 0.0	3.3 ± 0.0	n.d.	0.0
10	kaempferol-3-rutinoside	0.7 ± 0.1	1.5 ± 0.2	n.d.	0.0
11	kaempferol-3-glucoside	0.5 ± 0.0	1.1 ± 0.0	n.d.	0.0
12	quercetin rhamnoside	2.6 ± 0.0	5.6 ± 0.1	5.2 ± 4.2	0.7
13	hesperidin	10.3 ± 0.7	34.0 ± 2.3	n.d.	0.0
				Total phenolics	**5.8**
				TOTAL HPLC	**74.5%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.13 Also see Table 4.11 n.q. – not quantified; n.d. – not detected

Table 4.29. Antioxidant activity and levels of individual phenolic compounds in clementine.

		Quantity		Antioxidant activity	
Peak	**Compound**	**µg/g**	**nmol/g**	**Trolox equiv.(nmol/g)**	**Percentage**
vitamin C		322.7 ± 3.8	1835.5 + 21.6	276 ± 22.5	**61.5**
Phenolic compounds					
1	caffeic acid- conjugate	1.0 ± 0.0	5.7 ± 0.2	7.0 ± 0.3	1.6
2	nobiletin	8.2 + 0.0	30.4 ± 0.1	14.3 ± 1.1	3.2
3	3,3',4',5,6,7,8-heptamethoxyflavone	15.1 ± 0.2	55.8 ± 0.6	27.1 ± 0.7	6.0
4	naringenin-rutinoside-hexoside	3.2 ± 0.1	10.6 ± 0.3	n.d.	0.0
5	eriocitrin (eridictyol-3-rutinoside)	3.1 ± 0.0	10.2 ± 0.2	3.1 ± 0.3	0.7
6	naringenin conjugate	3.6 ± 0.1	12.0 ± 0.2	4.1 ± 0.4	0.9
7	quercetin-3-rutinoside	0.8 ± 0.1	1.7 ± 0.2	n.d.	0.0
8	narirutin (naringenin-3-rutinoside)	91.6 ± 0.4	303.3 ± 1.2	n.d.	0.0
9	hesperidin (hesperitin-3-rutinoside)	299.5 ± 4.3	991.7 ± 14.1	n.d.	0.0
10	hesperitin conjugate	6.0 ± 0.1	19.9 ± 0.2	n.d.	0.0
11	neoponcirin (isosakuranetin-3-rutinoside)	12.4 ± 0.1	41.1 ± 0.4	n.d.	0.0
				Total phenolics	12.4
				TOTAL HPLC	**74.9%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.14 Also see Table 4.12 n.q. – not quantified; n.d. – not detected

Table 4.30. Antioxidant activity and levels of individual phenolic compounds in green pepper.

		Quantity		Antioxidant activity	
Peak	**Compound**	**µg/g**	**nmol/g**	**Trolox equiv.(nmol/g)**	**Percentage**
Vitamin C		1137.3 ± 24.6	6461.8 ± 139.7	1559.6 ± 45.7	**88.8%**
Phenolic compounds					
1	caffeic acid hexose conjugate	4.39 ± 0.35	24.4 ± 1.9	n.d.	0.0
2	caffeic acid hexose conjugate	0.80 ± 0.04	4.4 ± 0.2	n.d.	0.0
	5-caffeoylquinic acid	n.q.	n.q.	n.d.	
3	quercetin-rhamnosyl-hexoside	1.38 ± 0.10	3.0 ± 0.2	n.d.	0.0
4	quercetin-3-glucoside	0.90 ± 0.04	1.9 ± 0.1	n.d.	0.0
5	luteolin-pentose-hexoside	1.03 ± 0.07	2.3 ± 0.2	n.d.	0.0
6	quercetin rhamnoside	28.03 ± 2.14	60.4 ± 4.6	34.7 ± 3.3	2.0
7	hesperidin	4.08 ± 0.23	13.5 ± 0.8	n.d.	0.0
8	luteolin-hexose-pentose-malonate	37.17 ± 2.41	83.0 ± 5.4	34.2 ± 2.7	1.9
				Total phenolics	**3.9**
Carotenoids and tocopherol					
1	unknown carotenoid (UV 450nm)	2.8 ± 0.0	5.2 ± 0.1	n.d.	0.0
2	unknown carotenoid (UV 450nm)	1.1 ± 0.0	2.0 ± 0.1	1.3 ± 0.3	0.1
3	lutein	1.2 ± 0.0	2.3 ± 0.0	n.d.	0.0
4	unknown carotenoid (UV 450-470 nm)	4.2 ± 0.0	7.8 ± 0.1	2.0 ± 0.2	0.1
5	α-tocopherol	0.7 ± 0.1	1.6 ± 0.1	3.1 ± 0.2	0.2
				Total carotenoids	**0.4**
				TOTAL HPLC	**94.0%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.15 Also see Table 4.13 n.q. – not quantified; n.d. – not detected

Table 4.31. Antioxidant activity and levels of individual phenolic compounds in yellow pepper.

		Quantity		Antioxidant activity	
Peak	Compound	µg/g	nmol/g	Trolox equiv.(nmol/g)	Percentage
Vitamin C		1122.4 ± 20.6	6377.3 ± 117.0	1251.8 ± 92.5	**88.0**
Phenolic compounds					
3	quercetin-pentose-dihexoside and quercetin-rhamnosyl-hexoside	0.3 ± 0.0	0.7 + 0.0	n.d.	0.0
7	hesperidin	3.8 ± 0.1	12.4 ± 0.4	n.d.	
8	luteolin-hexose-pentose-malonate	10.9 ± 0.1	24.3 ± 0.2	14.2 ± 1.5	1.1
				Total phenolics	**1.1**
Carotenoids and tocopherol					
1	unknown carotenoid (UV 450nm)	3.8 ± 0.3	7.1 ± 0.5	n.d.	0.0
2	unknown carotenoid (UV 430nm)	0.6 ± 0.1	1.1 ± 0.1	3.6 ± 0.4	0.3
3	unknown carotenoid (UV 450nm)	2.1 ± 0.1	3.8 ± 0.1	n.d.	0.0
4	lutein	5.4 ± 0.0	10.0 ± 0.0	3.1 ± 0.4	0.2
5	unknown carotenoid (UV 450-470nm)	2.0 ± 0.1	3.7 ± 0.2	0.6 ± 0.3	0.0
6	β-cryptoxanthine	0.2 ± 0.0	0.4 ± 0.0	2.1 ± 1.1	0.2
7	α-tocopherol	8.1 ± 1.2	62.2 ± 9.4	10.5 ± 5.2	0.8
				Total carotenoids	**4.9**
				TOTAL HPLC	**94.0%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.16 Also see Table 4.14 n.q. – not quantified; n.d. – not detected

Table 4.32. Antioxidant activity and levels of individual phenolic compounds in red pepper.

Peak	Compound	Quantity µg/g	Quantity nmol/g	Antioxidant activity Trolox equiv.(nmol/g)	Antioxidant activity Percentage
Vitamin C		1667.8 ± 14.4	9476.1 ± 82.1	1675.2 ± 11.9	69.8
Phenolic compounds					
3	quercetin-pentose-dihexoside quercetin-rhamnosyl-hexoside	0.9 ± 0.1	1.9 ± 0.2	n.d.	
6	quercetin-rhamnoside	1.1 ± 0.1	2.4 ± 0.2	n.d.	0.0
7	hesperidin	10.1 ± 3.2	33.3 ± 10.6	n.d.	0.0
8	luteolin-hexose-pentose-malonate	7.9 ± 0.7	17.6 ± 1.6	14.3 ± 2.6	0.6
				Total phenolics	**0.6**
Carotenoids and tocopherol					
1	unknown carotenoid (UV 450nm)	108.2 ± 0.5	201.6 ± 0.9	n.d.	0.0
2	unknown carotenoid (UV 450-470 nm)	41.9 ± 0.3	78.1 ± 0.5	8.2 ± 0.6	0.3
3	unknown carotenoid (UV 450-470 nm)	88.9 ± 0.4	165.7 ± 0.8	23.0 ± 2.6	1.0
4	unknown carotenoid (UV 450 nm)	217.8 ± 1.9	405.7 ± 3.5	79.5 ± 3.5	3.3
5	unknown carotenoid (UV 470 nm)	119.8 ± 42.5	223.2 ± 79.1	95.3 ± 12.5	4.0
6	unknown carotenoid (UV 450-470 nm)	73.3 ± 0.3	136.6 ± 0.5	n.d.	0.0
7	unknown carotenoid	15.5 ± 7.7	28.9 ± 14.3	n.d.	0.0
8	unknown carotenoid (UV 470 nm)	20.8 ± 9.4	38.8 ± 17.6	n.d.	0.0
9	β-cryptoxanthin (UV 450-470 nm)	22.8 ± 0.3	42.5 ± 0.5	53.3 ± 5.7	2.2
10	unknown carotenoid (UV 470 nm)	24.7 ± 0.5	46.1 ± 0.9	n.d.	0.0
11	α-tocopherol (UV 300 nm)	194.3 ± 3.5	451.2 ± 8.2	260.2 ± 14.9	10.8
12	unknown carotenoid (UV 460 nm)	16.3 ± 0.3	30.4 ± 0.5	n.d.	0.0
13	unknown carotenoid (UV 450-470 nm)	19.8 ± 0.4	36.9 ± 0.7	n.d.	0.0
14	unknown carotenoid (UV 470 nm)	28.5 ± 0.3	53.0 ± 0.5	n.d.	0.0
15	unknown carotenoid (UV 460 nm)	46.8 ± 3.9	87.1 ± 7.2	15.8 ± 1.7	0.7
16	unknown carotenoid (UV 470 nm)	36.9 ± 0.5	68.8 ± 0.9	n.d.	0.0
17	tocopherol (UV 300 nm)	44.7 ± 4.7	103.7 ± 10.8	50.0 ± 7.3	2.1
				Total carotenoids	**22.3**
				TOTAL HPLC	**92.8%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.17 Also see Table 4.15 n.q. – not quantified; n.d. – not detected

Table 4.33. Antioxidant activity and levels of individual phenolic compounds in cherry tomato.

		Quantity		Antioxidant activity	
Peak	**Compound**	**µg/g**	**nmol/g**	**Trolox equiv.(nmol/g)**	**Percentage**
Vitamin C		360.6 ± 25.0	2048.6 ± 141.8	422.6 ± 6.6	**58.2**
Phenolic compounds					
1	caffeic acid hexose conjugate	16.1 ± 0.1	89.2 ± 0.8	n.d.	0.0
2	caffeic acid hexose conjugate	8.2 ± 0.1	45.6 ± 0.5	n.d.	0.0
3	3-caffeoylquinic acid	4.4 ± 0.1	24.7 ± 0.5	27.2 ± 0.0	3.7
4	quercetin-3-rutinoside-7-glucoside	1.4 ± 0.1	3.0 ± 0.1	n.d.	0
5	5-caffeoylquinic acid	0.8 ± 0	4.3 ± 0.2	n.d.	0
6	quercetin-rutinoside-pentoside	7.8 ± 0.0	16.7 ± 0.1	13.5 ± 0.0	1.9
7	quercetin-3-rutinoside	66.4 ± 0.2	143.2 ± 0.4	45.7 ± 0.0	6.3
8	kaempferol-rutinoside-pentoside	1.0 ± 0.0	2.2 ± 0.0	n.d.	0.0
9	kaempferol-3-rutinoside	2.6 ± 0.0	5.8 ± 0.1	6.8 ± 0.0	0.9
10	4,5-dicaffeoylquinicacid	0.6 ± 0.0	3.4 ± 0.0	18.3 ± 0.4	2.5
11	tricaffeoylquinic acid	2.2 ± 0.1	12.1 ± 0.6	25.8 ± 0.4	3.6
12	naringenin	n.q	n.q.	**Total phenolics**	**18.9%**
				TOTAL HPLC	**77.1%**

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.18 Also see Table 4.16 n.q. – not quantified; n.d. – not detected

Table 4.34. Antioxidant activity and levels of individual phenolic compounds in plums.

		Quantity		Antioxidant activity	
Peak	Compound	ug/g	nmol/g	Trolox equiv.(nmol/g)	Percentage
Vitamin C		2.9 ± 0.0	16.3 ± 0.2	nd	0
Phenolic compounds					
1	procyanidin dimer	85.5 ± 2.9	294.7 ± 9.9	169.2 ± 21.2	5.8
2	procyanidin polymer	33.5 ± 2.7	115.4 ± 9.2	56.0 ± 8.0	1.9
3	(+)-catechin	105.5 ± 12.2	363.8 ± 41.9	217.7 ± 31.5	7.4
4	procyanidin dimer	49.4 ± 2.8	170.5 ± 9.6	96.7 ± 4.8	3.3
5	procyanidin pentamer	31.8 ± 2.0	109.8 ± 7.0	55.2 ± 2.0	1.9
6	procyanidin trimer	66.7 ± 7.7	230.1 ± 26.4	245.9 ± 34.8	8.4
	cyanidin-3-glucoside	52.6 ± 0.8	117.4 ± 1.8	n.d.	0.0
7	procyanidin dimer	23.2 ± 1.1	79.9 ± 4.0	39.0 ± 3.6	1.3
8	procyanidin polymer	15.8 ± 7.4	54.5 ± 25.4	75.7 ± 28.7	2.6
	cyanidin-3-rutinoside	19.6 ± 0.2	43.8 ± 0.5	n.d.	0.0
9	procyanidin dimer	90.9 ± 4.1	313.5 ± 14.0	208.5 ± 21.4	7.1
10	procyanidin hexamer	148.8 ± 104.9	513.1 ± 361.8	59.2 ± 0.8	2.0
11	(-)-epicatechin	103.6 ± 5.5	357.3 ± 19.0	209.3 ± 21.7	7.1
12	procyanidin polymer	29.5 ± 1.1	101.7 ± 3.8	19.2 ± 1.4	0.7
13	procyandin polymer	34.9 ± 1.6	120.2 ± 5.5	18.3 ± 1.4	0.6
14	catechin dimer	66.0 ± 2.8	227.3 ± 9.6	110.9 ± 8.5	3.8
15	procyandin polymer	83.2 ± 0.6	287.0 ± 2.2	83.8 ± 3.5	2.9
16	procyandin polymer	21.5 ± 1.3	74.2 ± 4.6	30.2 ± 1.6	1.0
17	procyandin polymer	76.6 ± 3.5	264.3 ± 12.2	104.5 ± 7.9	3.6
18	procyandin polymer	22.2 ± 1.1	76.5 ± 3.7	23.3± 3.9	0.8
19	procyanidin hexamer	61.7 ± 2.5	212.9 ± 8.7	71.8 ± 6.4	2.5
20	procyandin polymer	39.0 ± 1.2	134.3 ± 4.0	20.8 ± 1.7	0.7
21	procyanidin hexamer	55.9 ± 0.8	192.8 ± 2.7	83.4 ± 9.3	2.8
22	quercetin-3-rutinoside	23.3 ± 0.6	50.1 ± 1.2	40.2 ± 3.9	1.4
23	procyanidin pentamer	16.7 ± 2.0	57.6 ± 6.9	22.4 ± 1.2	0.8
24	procyanidin dimer	10.1 ± 0.7	21.7 ± 1.5	32.5 ± 2.8	1.1
25	quercetin-3-glucoside	39.7 ± 0.4	85.7 ± 0.8	45.8 ± 6.4	1.6
26	quercetin-pentose	4.1 ± 0.5	8.8 ± 1.2	6.4 ± 3.3	0.2
27	quercetin-pentosyl-pentoside	2.3 ± 0.3	5.0 ± 0.7	0.9 ± 0.0	0.2
28	quercetin-pentose	37.8 ± 0.9	81.4 ± 2.0	45.1 ± 4.3	1.5
29	quercetin-3-rhamnoside	6.5 ± 0.1	13.9 ± 0.3	5.4 ± 3.0	0.2
30	quercetin-malonate-hexoside	9.4 ± 0.1	20.3 ± 0.3	6.4 ± 3.3	0.2
				Total phenolics	73.3

Quantitative estimates expressed as mean values ± standard error (n = 3)

[a] Peak numbers and retention times refer to HPLC traces in Figure 4.19 Also see Table 4.17 n.q. – not quantified; n.d. – not detected

CHAPTER 5. INVESTIGATION OF THE ABSORPTION OF ANTHOCYANINS AND PROANTHOCYANIDINS FROM REDCURRANTS USING AN ILEOSTOMY MODEL 173

Chapter 5. Investigation of the absorption of anthocyanins and proanthocyanidins from redcurrants using an ileostomy model

Before conclusions can be drawn on the potential *in-vivo* effects of phenolics on human health, a more complete understanding of the mechanisms of absorption, bioavailability and biotransformations occurring is necessary.

To investigate the absorption and excretion of anthocyanins and proanthocyanidins in particular, a healthy subject with an ileostomy was fed 200 g of redcurrants and subsequently provided plasma, urine and ileostomy fluid for analysis.

5.1. Analysis of redcurrants

Phenolic compounds were extracted from freeze-dried redcurrants three times with acidified methanol (containing 0.1% HCl). The residue was acid hydrolysed to release the non-extractable tannins (prodelphinidins).

5.1.1. Analysis of redcurrant extract

Redcurrants were analysed by reversed-phase HPLC using a 60 min gradient: 5 % acetonitrile in 1% formic acid isocratic during the first 10 min, and 5 to 30 % over 50 minutes, with column eluate first directed to a photodiode array monitor then to a mass spectrometer with an electrospray interface operating in full scan MS^n mode. Samples were analysed with the mass spectrometer operating in negative and positive ionisation modes. The identification of phenolic compounds by HPLC, corresponding to peaks 1-17 in Figure 5.1, based on MS^2 and MS^3 data, as well as λ_{max}, are summarised below and presented in Table 5.1. The quantification of each compound is presented in Table 5.2. After identification of the phenolics in the berry extract by HPLC-PDA and MS^n detection, the extracts were further analysed with the post-column $ABTS^{\cdot+}$ antioxidant detector system (see Figure 5.1).

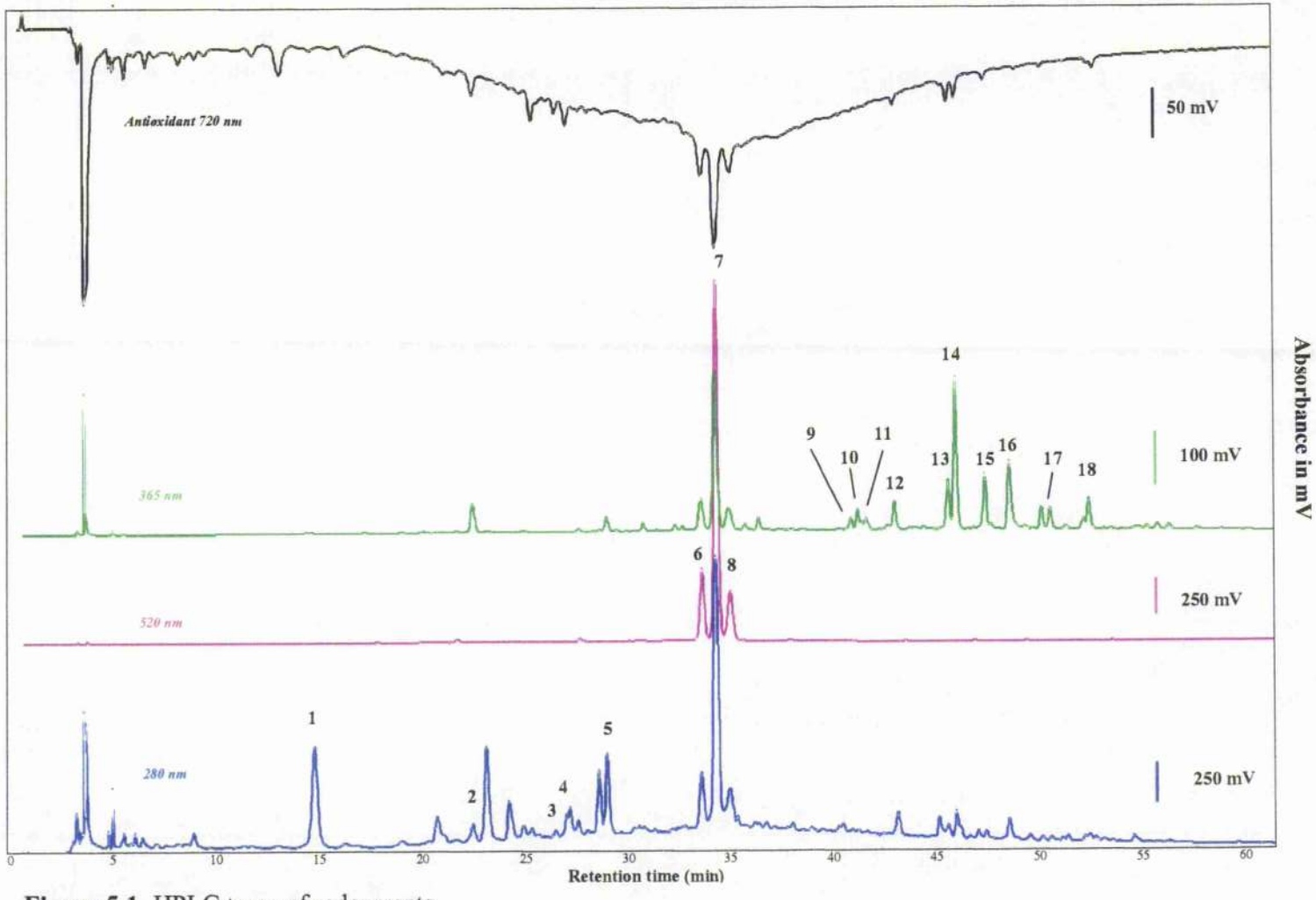


Figure 5.1. HPLC trace of redcurrants

Table 5.1. Summary of the phenolic compounds in an extract of redcurrants detected by HPLC with diode array and MS^2 detection[a]

peak	t_R	Compound	Absorbance (nm)	$[M-H]^-$ *m/z*	MS^2 ions (*m/z*)	MS^3 ions (*m/z*)
1	17.5	4-hydroxy-benzoic acid-hexose	260	299	179, 137 [HbenzAc]([M-H]$^-$ - Hex)	
2	22.8	caffeic acid-hexose conjugate	330	341	179 [CafAc]([M-H]$^-$ - Hex)	
3	23.8	caffeic acid-hexose conjugate	330	341	179 [CafAc]([M-H]$^-$ - Hex)	
4	27.0	caffeic acid-hexose conjugate	330	341	179 [CafAc]([M-H]$^-$ - Hex)	
5	28.5	*p*-coumaric acid hexose conjugate	315	325	163 [CoumAc]([M-H]$^-$ - Hex), 145	
6	29.2	cyanidin-3-sambubioside	515	581*	287[Cyan]([M+H]$^+$- Samb)	
7	30.1	cyanidin-3-xylosyl-rutinoside	510	727*	581([M−H]$^+$- Rham), 287 [Cyan]([M+H]$^+$- Xyl-Rut	
8	30.5	cyanidin-3-rutinoside	515	595*	449([M+H]$^+$- Rham), 287[Cyan]([M+H]$^+$- Glc-Rham)	
		cyanidin-3-sophoroside		611*	287[Cyan]([M+H]$^+$- Glc-Glc)	
9	37.5	myricetin-rutinoside	355	625	317 [Myr]([M-H]$^-$- Rut), 271, 242	
10	38.1	quercetin-pentosyl-rutinoside	350	741	609([M-H]$^-$- Pent, 301[Q]([M-H]$^-$ Pent-Rut), 271	
11	38.2	myricetin hexoside	350	479	317 [Myr]([M-H]$^-$- Hex)	
12	39.7	quercetin-pentosyl-rutinoside	350	741	609([M-H]$^-$- Pent, 301[Q]([M-H]$^-$ Pent-Rut), 271	
13	42.0	myricetin-rhamnoside	355	463	317 [Myr]([M-H]$^-$- Rham)	
14	41.9	quercetin-3-rutinoside	330	609	301 [Q]([M-H]$^-$- Rut)	271, 179, 255
15	43.3	quercetin-3-glucoside	350	463	301 [Q]([M-H]$^-$- Glc),	271, 179, 255
16	45.9	kaempferol-rutinoside	350	593	285 [K]([M-H]$^-$- Rut),	
17	47.5	kaempferol-3-glucoside	330	447	285[K]([M-H]$^-$- Glc)	
18	47.9	quercetin-rhamnoside	345	447	301[Q]([M-H]$^-$- Rham)	

HbenzAc, 4-hydroxybenzoic acid; Hex, hexosyl; CafAc, Caffeic acid; Cyan, cyanidin; Samb, sambubiosyl; Rut, rutinosyl; Xyl, xylosyl; Myr, myricetin; Rham, rhamnosyl; Q, quercetin; Glc, glucosyl; Mal, malonyl; K, kaempferol; t_R, retention time

[a] Peak numbers and retention times refer to HPLC traces in Figure 5.1 * positive ionisation molecular ion ($[M+H]^+$)

Table 5.2. Level of phenolic compounds in redcurrants

Peak	Compound	Quantity (µg/g)	Quantity in mg/200g serving
Benzoic acid			
1	4-hydroxy-benzoic acid-hexose	10.3 ± 0.3	2.1 ± 0.1
2	caffeic acid-hexose conjugate	3.0 ±0.2	0.6 ± 0.0
3	caffeic acid-hexose conjugate	1.0 ±0.0	0.2 ± 0.0
4	caffeic acid-hexose conjugate	2.5 ± 0.6	0.5 ± 0.1
5	*p*-coumaric acid hexose conjugate	13.5 +1.9	2.7 ± 0.4
Total		**30.3**	**6.0**
Anthocyanins			
6	cyanidin-3-sambubioside	23.2 ± 1.2	4.6 ± 0.2
7	cyanidin-3-xylosyl-rutinoside	75.0 ± 6.6	15 ± 1.3
8	cyanidin-3-rutinoside/ cyanidin-3-sophoroside	24.2 ± 1.6	4.8 ± 0.3
Total		**122.4**	**24.5**
Flavonols			
9	myricetin-rutinoside	1.8 ± 0.1	0.4 ± 0.0
11	myricetin hexoside	8.0 ± 0.1	1.6 ± 0.0
13	myricetin-rhamnoside	1.7 ± 0.0	0.3 ± 0.0
	Total myricetin	**11.5**	**2.3**
10	quercetin-pentosyl-rutinoside	2.1 ± 0.2	0.4 ± 0.0
12	quercetin-pentosyl-rutinoside	6.1 ± 0.1	1.2 ± 0.0
14	quercetin-3-rutinoside	26.1 ± 0.4	5.2 ± 0.1
15	quercetin-3-glucoside	12.2 ±1.8	2.4 ± 0.3
18	quercetin-rhamnoside	2.9 ± 0.2	0.6 ± 0.0
	Total quercetin	**49.4**	9.9
16	kaempferol-rutinoside	4.6 ± 0.0	0.9 ± 0.0
17	kaempferol-3-glucoside	1.5 ± 0.3	0.3 ± 0.0
	Total kaempferol	**6.1**	**1.2**
Total		**66.9**	**13.4**
Prodelphinidins (after acid hydrolysis)		**264.5 ± 5.3**	**52.9 ± 1.1**

Peak numbers and retention times refer to HPLC traces in Figure 5.1

5.1.2. Analysis of redcurrants residue

When subjected to acid hydrolysis, strong purple colours were formed in the residue of the redcurrant extract, due to the release of delphinidin pigments from colourless prodelphinidin polymers.

Prodelphinidins were quantified in delphinidin equivalents, using the HPLC-MS system. Results are presented in Table 5.2. In a previous study Maatta *et al.* (2001) reported the presence of 34 µg/g of proanthocyanidins, after acid hydrolysis of the residue using less acidic conditions (HCl 0.6 M in methanol at 60°C for 2h). In the present study, the presence of 264 µg/g delphinidin was detected after acid hydrolysis (HCl 2M at 90°C for 2h). This difference could be due to a more efficient conversion of prodelphindins to the corresponding delphinidin under our acid hydrolysis conditions.

The antioxidant potential of both redcurrant extract and acid hydrolysed residue was measured using the FRAP assay. Results are presented in Figure 5.2.

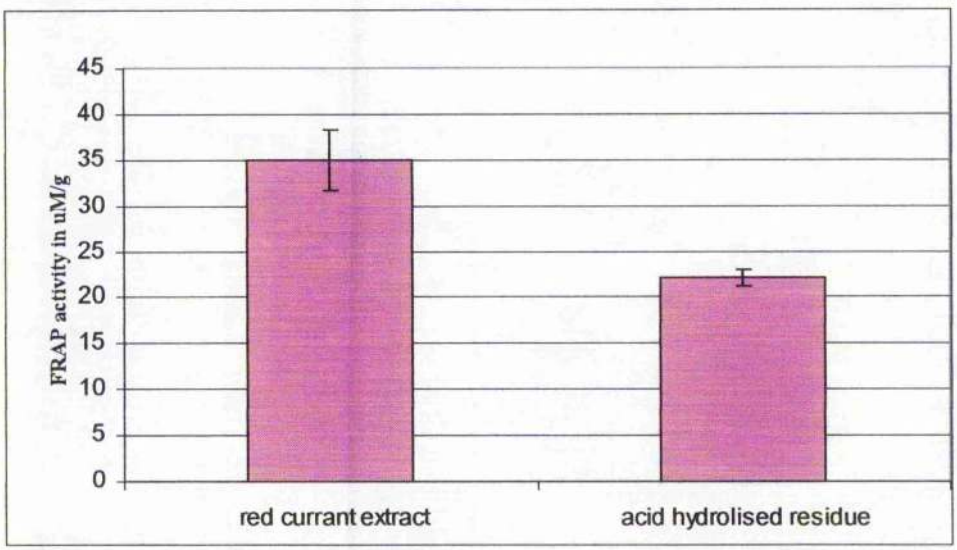


Figure 5.2. Antioxidant activity of redcurrant extract and hydrolysed residue by FRAP assay

The acid hydrolysed extract exhibited high antioxidant activity after the transformation of prodelphinidins to delphinidin monomers. Measurement of the direct antioxidant activity of prodelphinidins was not possible, as they were not extractable, but results suggest that prodelphinidins are a potential source of strong antioxidants in redcurrants. Proanthocyanidins are known to have antioxidant properties (Santos-Buelga and Scalbert, 2000) but the degree of polymerisation and the nature of units constituting polymeric proanthocyanidins seems to have an impact (Saint-Cricq de

Gaulejac *et al.*, 1999). The scavenging capacity increased up to the trimers or tetramers and then decreased for larger polymers.

5.1.3. Conclusions

Redcurrant extracts contained a range of phenolic compounds including flavonols, benzoic acids and anthocyanins. The anthocyanins, in particular cyanidin-3-xylosyl-rutinoside were the main contributors to the antioxidant activity, as shown by the on-line ABTS system. Redcurrants also contained some non-extractable prodelphinidins, probably bound to the cell wall, suggesting a high degree of polymerisation. These tannins are also a potential source of strong antioxidants. What happens to these compounds *in vivo*?

5.2. Analysis of biological samples

An ileostomy subject followed a reduced flavonoid diet for 2 days prior to the study. On day 3, after an overnight fast, the subject consumed 200 g of redcurrants with some low fat yoghurt. Venous blood samples were collected into heparinised tubes up to 24 hours following redcurrant consumption. Subjects provided a sample of urine and ileal fluid prior to redcurrant consumption and in three time periods 0-2 h, 2-5 h and 5-24 h following supplementation.

5.2.1. Plasma analysis

Plasma samples were extracted using the method of Day *et al.* (2001) and analysed with an HPLC-MS^n system, using a 60 min, 5-40% acetonitrile gradient. Samples were analysed with the mass spectrometer operating in fullscan negative and positive ionisation modes. Neither anthocyanins or their possible conjugates were detected in plasma following the consumption of redcurrants.

5.2.2. Urine analysis

Urine samples were concentrated 10 times, using a rotovapor, before being analysed the same way as plasma samples by HPLC-MS^n. Low levels of intact and unmetabolized cyanidin-3-xylosyl-rutinoside (5.3 ng in total, corresponding to 0.02 % of the total anthocyanin ingested) was excreted in urine between 2 and 5h following the consumption of redcurrants. This was in agreement with McGhie *et al.* (2003), who

reported that anthocyanins containing different core aglycones were excreted in urine without modification or metabolism. As a percentage of the amount consumed, less than 0.1% was excreted.

5.2.3. Ileal fluid analysis

Ileostomy fluid homogenate samples were extracted three times using 50% aqueous MeOH in 1% formic acid, before analysis by HPLC-PDA-MS^n system using a 60 min, 5-40% acetonitrile gradient. The residue was subjected to acid hydrolysis, in order to quantify the non-extractable prodelphinidins. Ileal fluid extracts were further analysed with the mass spectrometer replaced with the post-column $ABTS^{\cdot+}$ antioxidant detector system (see Figure 5.3).

A range of cyanidin conjugates were identified and quantified in ileal fluid, all present in the unmodified form. Quercetin-rhamnoside and myricetin rhamnoside were also identified in ileal fluid. Results are presented in Table 5.3. As shown in Figure 5.4, most of the ileal excretion occurred between 5-24 h. Analysis of ileal fluid samples 5-24 h after the redcurrant meal showed that cyanidin-3-xylosyl-rutinoside and cyanidin-3-rutinoside significantly contributed to the antioxidant activity of the ileal fluid. Prodelphinidins were also excreted during this period.

5.2.4. Discussion

Plasma anthocyanins were not detected after the consumption of 200 g of redcurrants; however they were detectable in low levels in urine, with the total amount excreted during 24 h being only 0.02% of the quantity consumed. This was in agreement with Wu *et al.* (2002). Cao *et al.* (2002) detected anthocyanins as glycosides in plasma, but this was after the consumption of 720 mg of anthocyanins (much more than the 24.5 mg ingested in the present study).

Absorption and excretion of anthocyanins differs from that of other phenolics as they appear to be mainly excreted unmetabolized in the glycosylated form in which they were administrated (Manach *et al.*, 2004). Nonetheless, Wu *et al.* (2002) reported the presence of lower levels of methylated and glucuronidated anthocyanin metabolites in urine from elderly women following ingestion of an elderberry extract.

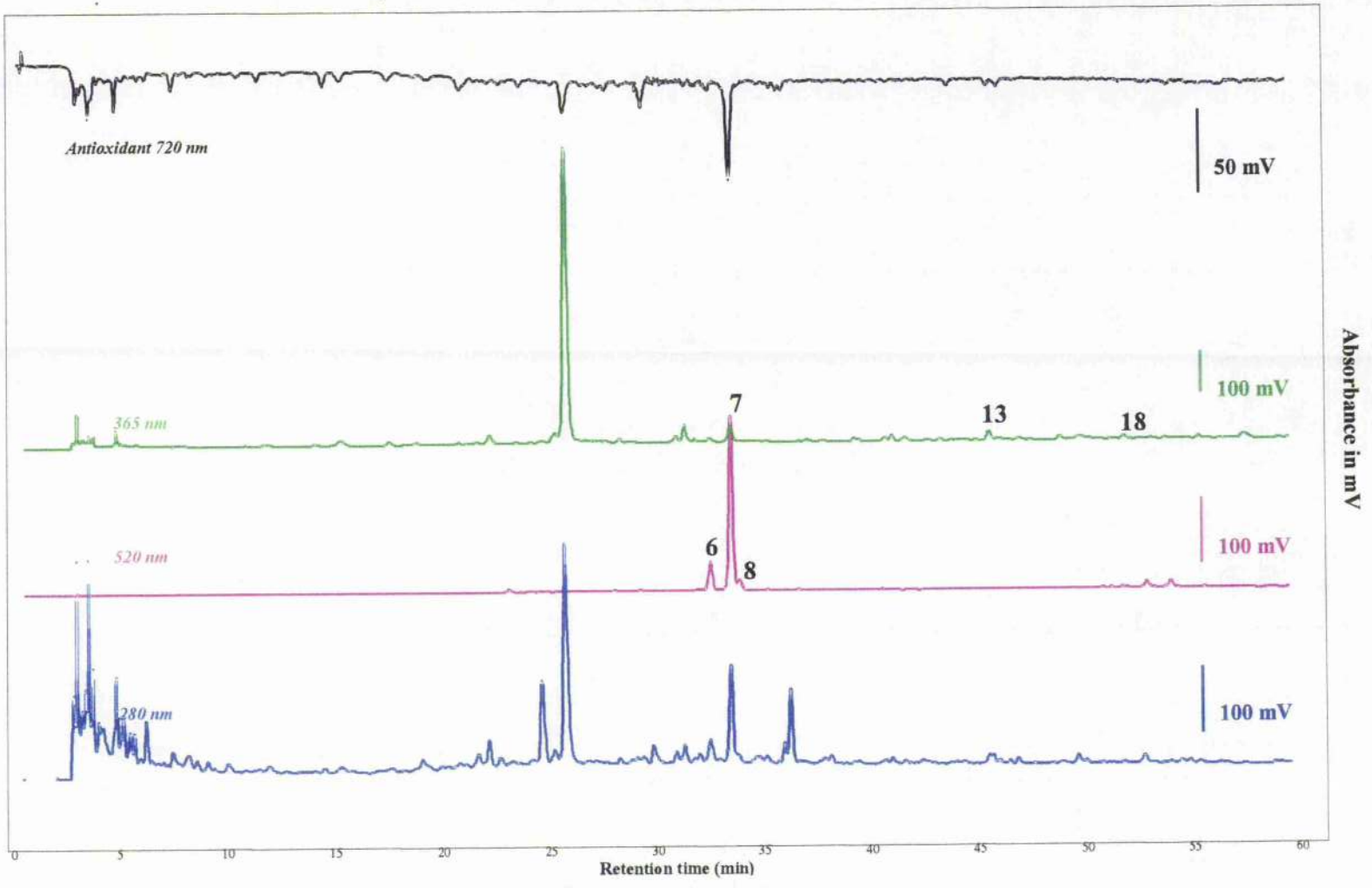
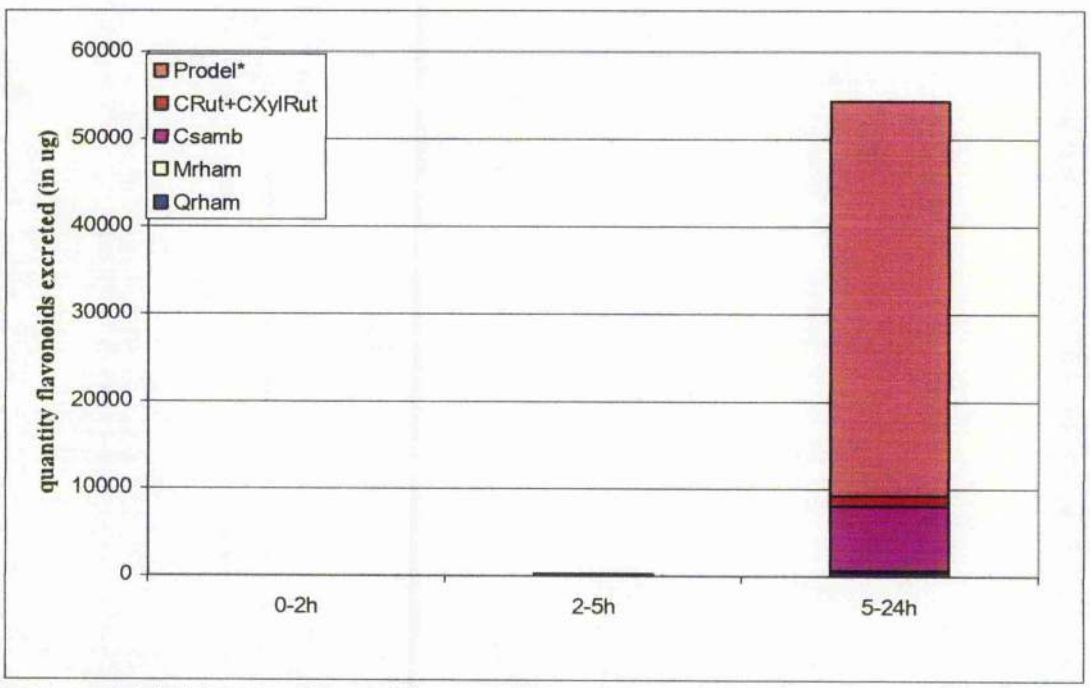


Figure 5.3. HPLC trace of ileal fluid 5-24h after consumption of redcurrants

Table 5.3. Quantification of phenolic compounds in ileal fluid and % excretion recovery after the consumption of 200 g of redcurrants

Compound	0h	0-2 h	2-5 h	5-24 h	% excreted over 24h
cyanidin-3-sambubioside	0	0	0	1165	25%
cyanidin-3-xylosyl-rutinoside/ cyanidin-3-rutinoside	0	0	0	7405	37%
Total cyanidin	0	0	0	8570	35.0
quercetin (quercetin-rhamnoside)	0	10	7	437	4.6
myricetin (myricetin-rhamnoside)	0	0	0	244	10.6
Prodelphinidins*	0	0	254	45414	85.8

*quantification (in µg) after acid hydrolysis



Prodel, prodelphinidins; Csamb, cyanidin-sambubioside; CXylRut, cyanidin-3-xylosyl-rutinoside; CRut, cyanidin-3-rutinoside; Mrham, myricetin-rhamnoside; Qrham, quercetin-rhamnoside
*quantification done after acid hydrolysis

Figure 5.4. Ileal fluid excretion of polyphenols (in µg) following the consumption of 200g of redcurrants

The anthocyanins, are excreted in urine in far lower concentrations than quercetin conjugates. Indeed, conjugated quercetin excreted in urine after onion consumption corresponded to 0.98% of the amount ingested (Aziz *et al.*, 1998), whereas for anthocyanins it was less than 0.1% (McGhie *et al.*, 2003), with one exception, the urinary excretion of pelargonidin from strawberries, which corresponded to 1.8 % of the amount ingested (Felgines *et al.*, 2003). This was accredited to analysing the urine immediately after collection as opposed to after storage of frozen samples, although we have been unable to substantiate this claim in test carried out in Glasgow (Borges *et al.*, unpublished data).

Ileostomy subjects have their colon surgically removed; therefore ingested food passes through the stomach and ileum, as it would in the intact gut and then passes directly into a collection bag outside the body. What would normal pass from the small to the large intestine is therefore recovered in the ileal fluid. The ileal model has the advantage that an excretion profile could be obtained, giving a time span for the absorption of redcurrant phenolics.

As only 35 % of anthocyanins were excreted intact in ileal fluid, the 65 % "missing" are likely to be absorbed or present in another form not detected in the current study. This is due to an incomplete understanding of their metabolism and also to an inadequate assessment of their *in vivo* forms, as anthocyanins and particularly the red flavylium cation form, are unstable. Indeed, depending on the pH, anthocyanins occur as an equilibrium of four molecular species: red flavylium cation at low pH (1-3), blue quinonoidal anion at pH 7-12, colourless pseudobase (for pH >3) and colourless chalcone (pH>3) (Clifford *et al.*, 2000). Further investigations are required to more fully understand the *in vivo* bioactivity of anthocyanins and to determine to what extent and in what form the "missing" anthocyanins are bioavailable and sequestered in body tissues.

Prodelphinidins are not efficiently absorbed in the small intestine, probably due to their high molecular weight. Indeed 86 % are recovered in ileal fluid, most probably in their intact form. Indeed Donovan *et al.* (2002) reported that proanthocyanidins having a degree of polymerization higher than three are not degraded in the acidic conditions of the stomach *in vivo*. Nonetheless, these compounds may not need to be

systemically absorbed to be protective, and they could exert local activity within the gastrointestinal tract, by acting as antioxidants. This local action may be very important because the gastrointestinal tract is particularly exposed to oxidizing agents and may be affected by inflammation, Crohn's disease, ulcerative colitis and cancer (Halliwell *et al.*, 2000). In this context, prodelphinidins could exert a significant protective effect, as substantial levels of unabsorbed prodelphinidins will pass through the intestine and the colon. This requires further investigation. In the colon, these compounds may be further degraded by the colonic microflora. Indeed Deprez *et al.* (2000) reported that procyanidins with an average level of polymerisation of six could be degraded into low molecular-weight aromatic acids, during 48 h *in vitro* incubation with human colonic microflora under anaerobic conditions. If such depolymerisation occurs for prodelphindins *in vivo*, and whether these aromatic acids are absorbed *in vivo* through the colon and act as protective molecules require further investigation.

Concerning the flavonols, 5 % and 10 % of the ingested amount of quercetin and myricetin respectively were excreted in the ileal fluid. Walle *et al.* (2000) reported that 19.5 %-35.2 % of total quercetin glycosides ingested in an onion meal were detected in ileal fluid. But in the case of redcurrants, the flavonol content is quite low and comprises 11 different flavonols. Excreted flavonols may be below the limit of detection. Furthermore, individual variations are quite large; previous work in our lab (unpublished data) showed excretion of flavonols following the consumption of onion soup varies between 6-35 %.

In the present study, most of the polyphenols (including prodelphinidins) were excreted in ileal fluid between 5 and 24 h following the consumption of redcurrants. In future, it could be interesting to determine the "real" peak in excretion, by collecting extra samples between 5 and 12 h for example.

5.3. Conclusions

The health effects of phenolic compounds depend on both their intake and their bioavailability, which can vary greatly. In the present study, we could see that prodelphinidins were poorly absorbed (as 86 % were recovered in the ileal fluid), and

their action in subjects with an ileostomy is thus restricted to the intestine. For anthocyanins, the same could be true, as less than 0.1 % of the ingested dose is excreted in urine (0.02 % in the present study, following the consumption of redcurrants). Yet the use of an ileal model suggested that 65 % of the anthocyanins were transformed and/or absorbed in humans, as only 35 % of the ingested dose was recovered in the ileal fluid. Further investigations are required:

i) to determine the nature of the "missing" anthocyanins, and their potential bioavailability and

ii) to determine if the non absorbed material (35 % anthocyanin, 85 % prodelphinidins) could have protective effect by acting as antioxidants within the gastrointestinal tract.

In this context, the use of radiolabeled anthocyanins for feeding studies in rat models could be very helpful to see more precisely what happens to the dietary anthocyanins after ingestion. Anthocyanins could then be traced among biological fluids, tissues and gastrointestinal tract and the nature of the metabolites or catabolites could be determined. Using this knowledge, further human feeding studies could be carried out (with more than one volunteer involved) and the choice of the feed could be improved. Indeed if redcurrants are particularly rich in prodelphinidins, other berries such as blackcurrants contain much higher levels of anthocyanins and are also a potent source of proanthocyanidins (Maatta *et al.*, 2004).

Chapter 6. Conclusions and perspectives

6.1. General discussion

There is now a considerable body of evidence showing that diets rich in fruits and vegetables are generally associated with lower disease risk. In the search for the specific bioactive components, attention has been focused on antioxidant compounds, initially carotenoids, vitamins E and C and more recently on other secondary plant-derivatives such as polyphenolic compounds, which may contribute to the alleged protective effect.

In the present study, by combining three different techniques (HPLC, MS and on-line measurement of antioxidant activity), we managed to identify the polyphenols present in a range of fruits and vegetables and determined their individual contribution to the total antioxidant activity. Results showed that in fruits and vegetables samples the total antioxidant activity is due to the cumulative effects of different compounds rather than any individual compound. The nature and quantity of antioxidants present varied among plant species. Certain polyphenols, such as flavonoids, are ubiquitous, present in most fruits and vegetables, in different levels, whereas others are specific to particular foods (e.g flavanones in citrus fruits). Therefore if the epidemiological evidence for the benefit of consuming diets rich in fruits and vegetables is quite compelling, the evidence for specific fruits or vegetables, and indeed specific compounds, is all but non-existent. These results underline the importance of a varied diet. Dietary advice to eat more fruits and vegetables should emphasize the advantage of variety rather than focusing on a particular type. In this context the current public health advice "try to eat five different fruits and vegetables each day" is quite relevant.

The levels of plant bioactive compounds recorded in the present study have to be considered in context and are not representative of a plant species. Indeed the content of individual fruits and vegetables is affected by many factors including variety, soil, climatic conditions, agricultural methods, degree of ripeness, storage conditions, physiology or stress under which they are grown.

Where agricultural practices are concerned, there is growing support for the belief that organic production may increase antioxidant levels in fruits and vegetables. In the present study we analysed organic and conventional varieties of fruits and vegetables available on the local market. Despite the interest of consumers in organic products, the findings from this study did not show any consistent differences in antioxidant capacity, vitamin C or phenolic content between conventional and organic crops obtained from commercial source. This is important from a consumer's point of view. These findings did not support the theory that organic produce are healthier than the conventional produce. To fully evaluate the two food production systems many other aspects such as environmental, economical and social factors may be considered.

If polyphenols in fruits and vegetables have antioxidant activity *in vitro*, issues relating to bioavailability and metabolism of the various polyphenols have to be considered. Indeed even if certain bioactive compounds give promising results *in vitro*, it does not mean they can perform similar functions in humans *in vivo*. These compounds could have a lower intrinsic activity, being poorly absorbed from the intestine, highly metabolized or rapidly eliminated. In addition, the metabolites found in plasma, urine or tissues may differ from the native substances in terms of biological activity. It is important in this respect to know to what extent the different dietary phenolics remain intact, what proportion are degraded by gut micro-organisms and enter the circulatory system or are recycled into the colon after systemic metabolism, and how much the transformation affects their bioactivity. In the present study, we could see that prodelphinidins were poorly absorbed (86 % of the ingested dose was recovered in the ileal fluid during the 24 h following the consumption of redcurrants), and their action is thus restricted to the intestine. Where anthocyanins are concerned, the present study showed than only 35 % of the total anthocyanins were excreted in the ileal fluid, and less than 0.01 % in urine. This suggested that 65 % of anthocyanins were transformed to another form, which we were not able to detect (absorbed or not) because they are either degraded in the gastrointestinal tract or absorbed and sequestrated in body tissues.

6.2. Future work and recommendations

More information is required on the bioavailability of antioxidants from fruits and vegetables in humans in order to establish whether the effects reported *in vitro* are relevant *in vivo*. In particular it could be useful to focus on anthocyanins, as these compounds are obviously transformed in the body into unidentified products. In this context, use of radiolabeled anthocyanins for feeding studies in rat models could be very helpful. Using this method, we could identify the fate of anthocyanins absorbed (if they reach and are deposited in body tissues), to what extent they are absorbed and above all in what form, before investigation of their potential activity *in vivo*.

Food composition tables, including all polyphenols, are required, as only partial data for certain polyphenols have been published on the basis of direct food analysis. It should allow calculation of daily polyphenol consumption from dietary questionnaires. Polyphenol intake could then be correlated with the incidence of certain diseases in epidemiologic studies, which would permit investigations of the protective role of these bioactive compounds.

The impact of the losses of potentially beneficial components resulting from storage, processing and cooking needs to be evaluated. Indeed onions and tomatoes lose between 75 % and 80 % of their initial quercetin content after boiling for 15 min and 30 % after frying (Crozier *et al.*, 1997). Loss of bioactive substances during cooking could be reduced by using the minimum of water (steaming or frying instead of boiling) (MacEvilly *et al.*, 2003). Are these losses similar for other polyphenols?

Regarding the growing number of polyphenol supplements available on the market, tests concerning the possible "adverse" effects of pharmacological doses of polyphenols are required. Indeed in France polyphenol supplements represent 15 to 20 % of the phytochemical market (Christen, 2002) even though, unlike the USA, health claims relating to polyphenols are not authorised. Examples of such products are numerous: Provinols, an extract of red wine containing 95 % of polyphenols commercialised by Seppic in 2002, powder containing 98 % oligomeric procyanidin marketed by "La Gardonnenque", Phytonutriance, an extract of apples rich in phloridzin. In addition to the

perceived protective effects of polyphenols and antioxidants against cardiovascular disease and some cancers, anti-aging properties of flavonoids are also promoted by industy.

Even although the active compounds have not yet been identified, agrofood cosmetic and pharmacologic companies flood the market with supplements rich in flavonoids. Polyphenols are promoted by industry more as a means to enhance sales of their products, than any concern over public health. Supplementation has to be considered very carefully. Indeed, in the past, an intervention study using a large dose of β-carotene, which was believed to have beneficial effects against cardiovascular disease, had to be stopped because of its dramatic negative effects on the health of certain groups (namely the heavy smokers). And polyphenols, aside from their antioxidant activity also have some anti-nutritional properties: polyphenols, and proanthocyanidins in particular, reduce the bioavailability of proteins and are likely inhibit non heam iron absorption (Santos-Buelag and Scalbert, 2000). Consequently it is advisable for population groups most susceptible to developing iron deficiency (infants, children, pregnant women,...) to avoid an excessive consumption of polyphenol rich beverages and foods or to avoid their consumption together with meals (Santos-Buelag and Scalbert, 2000). Furthermore, the bioavailability of supplements has to be considered, as the food matrix could have a major impact on the availability of polyphenols. The conclusions of the COMA Working Group on cancer do not support the view that either vitamin supplements or fortified foods provide an alternative to increasing the consumption of fruits and vegetables.

Yet polyphenols are unlikely to be the miracle ingredients, which will resolve the problems of the typical illnesses of western society. Indeed, if polyphenols are highlighted, we should keep in mind that other nutritional factors could also contribute to the "protective" effect of fruits and vegetables: they are indeed generally low in fat, may contain other vitamins and minerals and dietary fibre. It is all a question of balance.

I personally believe that a balanced diet is a healthier option than junk foods with supplements!

Chapter 7. List of references

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